

**CHARACTERISTICS OF TEA PRODUCED FROM YOUNG UPLAND RICE
LEAVES OF DAMGATONDAM CULTIVAR**



**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT
FOR THE DEGREE OF DOCTORAL OF PHILOSOPHY
IN FOOD SCIENCE
FACULTY OF AGRO-INDUSTRY
KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG**

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ไม่ว่ากรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ดัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้



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Thesis	Characteristics of tea produced from young upland rice leaves of Damgatondam cultivar
Student	Miss Narissara Uthai
Student ID	57608005
Degree	Doctor of Philosophy
Program	Food Science
Year	2019
Advisor	Assistant Professor Dr. Soraya Kerdpi boon

ABSTRACT

Green tea was made from one-month old leaves of the Thai upland black waxy rice cultivar 'Damgatondam' (DGTD), which has a pleasant smell of jasmine. The objective was to determine the optimum process in this experiment required to produce green tea that is acceptable to consumers and has health benefits. The processing conditions were steaming at $98 \pm 2^\circ\text{C}$ for 30, 45, 60, 75, 90 or 105 sec then pan roasting at $70 \pm 2^\circ\text{C}$ for 5, 10, 15, 20, 25 or 30 min and finally drying in an electrical cabinet tray dryer at $60 \pm 2^\circ\text{C}$ for 30, 45, 60 or 90 min. Brewed samples were compared to brewed samples of seven commercially available *Camellia sinensis* tea products by descriptive analysis with 10 trained panelists. Samples were also indentified the volatile compounds and determined the propoty changes during storage. It was found that the conditions that produced the highest quality of DGTD tea were: steaming at $98 \pm 2^\circ\text{C}$ for 60 sec, which completely inactivated peroxidase enzymes and preserved the green color, followed by pan roasting at $70 \pm 2^\circ\text{C}$ for 30 min and tray drying at $60 \pm 2^\circ\text{C}$ for 90 min in order to achieve a moisture content of less than 5%. The proximate analysis showed that DGTD tea prepared by the above method had: 22.59% crude fiber, 17.73% protein, 7.94% ash 3.77% moisture content and 2.58% fat. K, Ca, Mg, Mn, Na and Fe levels were 20400, 3400, 3200, 139.1, 113.8 and 66.4 mg/kg, respectively. The protein, fat, crude fiber, ash, Mg, K and Na contents in DGTD tea were higher than levels in tea from *Camellia sinensis*. Total phenolic content of DGTD tea was 9.53 mg GAE/g and antioxidant activity was 357.52 mM TE/g by ferric reducing antioxidant power (FRAP), 134.08 mM TE/g by 2,2 diphenyl-1-picrylhydrazyl assay (DPPH) and 108.89 mM TE/g by 2,2'-azino-bis (3-ethylbenzothiazo line-6-sulphonic acid) (ABTS), respectively. The descriptive analysis by trained panels identified 11 particular attributes of DGTD tea, which were yellowness, clearness, tea odor, hay odor,

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green odor, seaweed odor, jasmine rice odor, tea flavor, seaweed flavor, bitter taste and astringent taste. The descriptive analysis showed that the DGTD tea was less bitter and less astringent than *Camellia sinensis* tea and its jasmine odor was positively accepted. Forty-eight volatile compounds were identified in DGTD tea, using headspace solid phase micro-extraction, and were grouped into classes of substances, including 14 hydrocarbons (29.16%), 9 aldehydes (18.78%), 7 acids (14.58%), 7 esters (14.58%), 6 ketones (12.50%), 2 aromatic organic compounds (4.16%), 2 alcohols (4.16%), and 1 phenolic compound (2.08%). All of which were found to be positive volatile compounds that provided positive fragrant odors.

Key words: Bioactive compounds, brewing method, Damgatondam tea, sensory attributes, storage time, volatile compound



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ACKNOWLEDGEMENTS

Firstly, I would like to acknowledge Rajamangala University of Technology Krungthep (RMUTK) for 3 years scholarship.

I would like to express my gratitude to my advisor, Asst. Prof. Dr. Porjai Thamakorn and Asst. Prof. Dr. Soraya Kerdpi boon for her patience, motivation and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. Moreover, I would like to thank Prof. Dr. Anthony Kieth Thompson for correcting the English.

I would like to thank Dr. Nathamol Chindapan for her guidance about the research of gas chromatography-mass spectrometry.

I would like to thank my thesis committees: Assoc. Prof. Dr. Wanna Tangcharoenchai, Assoc. Prof. Dr. Sontisuk Teerachaichayut, Dr. Sawaminee Nualkeakul and Dr. Pajaree Ingkasupart for their insightful comments and encouragement, but also for the hard questions which improve my research.

I also thank Miss Thitiya Sonthirawd, labmate and other lovely friends for her kindness and helpfulness.

I would like to thank my family, my parents for their supporting and encouragement.

Finally, I would like to thank professors, lecturers, officers, laboratory and technical staffs at Agro-Industry KMITL for their teaching, training and assisting.

NARISSARA UTHAI

2019

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CHAPTER 1

INTRODUCTION

Tea is mostly made from the young leaves and a bud of *Camellia sinensis*, which are fermented to produce black tea or not fermented to produce green tea (Cabrera *et al.*, 2006). Green tea is produced by inactivating polyphenol oxidase in the terminal 2 leaves and bud by applying either heat or steam before drying. There are many other plants used to produce tea including mulberry leaves, pandanus leaves, safflower and chamomile flower. These kinds of tea are mainly produced by the same process as that used for *C. sinensis* green tea.

Thailand is a major world producer of rice both for local consumption, where it forms the major staple, and for export. Most rice is irrigated but some is produced, mainly by small farmers, without irrigation called upland rice or hill rice (Somrit, 1988). Having a by-product from the young plants (rice grass) could enhance the income of hill rice farmers. Hill rice is grown from seed either by direct seeding or by transplanting. In both cases, young seedlings are removed and many are discarded. These young seedlings could be utilized to provide an additional income for farmers.

New pure-line selections of local upland fragrant rice cultivars in Thailand has resulted in many new varieties with particular characteristics, including Damgatondam (DGTD), which has a distinct jasmine aroma in both seeds and leaves (Nokkoul, 2016). Green tea has previously been reported to be successfully produced from 14-21 day old leaves from 3 commercial fragrant rice cultivars (Khao Dawk Mali 105, Pathum Thani 1 and Sakon Nakhon) that had compositions of 5.50-6.05% moisture, Ca 3.7-4.2 mg/kg, Na 204.0-316.0 mg/g, K 11.8-19.3 g/kg, Mg 1.9-4.2 g/kg and Fe 66.0-110 mg/kg (Sudtasarn *et al.*, 2008). Khanthapok *et al.* (2015) compared the qualities of juice squeezed from seven colored rice grasses (Kum Doisaket, Kum Ka, Kum Noi, Kum Pe, Kum Ton Khieaw, Niaw Dum Chor Mai Phi and Riceberry) and seven white rice grasses (Khai Mod Rin 3, Khao Dawk Mali 105, Khao Gaw Diaw 35, Leb Nok Pattani, Pathum Thani 1, Plai Ngahm Prachin Buri and RD6) compared with Fang 60 as wheatgrass (*Triticum aestivum*). The result showed that the colored (purple) rice grass juices exhibited greater antioxidant potential than the grass juices from white rice and wheatgrass. Yun (2016) successfully made freeze dried rice grass juice from samples of 4 weeks old DGTD leaves and showed

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that it had abundant bioactive compounds and high antioxidant activity as well as retaining its characteristics jasmine aroma.

From the previous researches, these benefits of color rice or rice grass are interesting and green tea also has abundant benefits. The production of green tea achieved from upland rice leaves could present the interesting characteristics to the products. However, there is none of the research develop green tea from DGTD upland rice grass leaves. Thus using these materials with the process from green tea to make DGTD tea is therefore interesting. Investigation the optimized steps in green tea process, bioactive compounds and antioxidant activity, brewing methods, volatile compound and storage time are presented.

1.1 Objectives

The objectives of this research were:

1. Study on process optimization, antioxidant activity and sensory characteristics of tea made from young DGTD rice grass leaves
2. Study on effect of the preparation and brewing methods on the bioactive compounds and sensory acceptability of DGTD tea
3. Identify volatile compounds of DGTD tea
4. Study on influence of storage time on physical, chemical, biological and sensory properties of DGTD tea

1.2 Scope of research

Thai upland black waxy rice grass leaves variety Damgatondam (DGTD) grown in Chumphon province, Thailand was used as material to produce tea using the same process as that used for green tea. Steaming for inactivating peroxidase enzyme was tested at 30, 45, 60, 75 or 90 sec. Pan roasting (first step of dehydration) at 70°C for 5, 15, 20, 25 or 30 min and tray drying (second step of dehydration) at 60°C for 30, 45, 60 or 90 min were studied. Total phenolic content and antioxidant activity by FRAP, DPPH and ABTS assays were determined. Moreover, effects of brewing parameters; i.e. tea weight (1, 1.5 and 2g) and water temperature (80, 90 and 98°C) on color, bioactive compounds, antioxidant activity and sensory acceptability of DGTD tea were studied. Sensory descriptive analysis of DGTD tea conducted by trained panelists was performed. Identification of volatile compound in DGTD tea by gas chromatography mass spectrometer using headspace-solid phase microextraction was studied. Determination of proximate analysis, several key minerals including Ca, Na, Mg, K, Fe, and Mn, total phenolic and antioxidant activities of

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DGTD tea product were analyzed. Effect of storage time on moisture content, water activity, A_w , color value, total phenolic content and antioxidant activity (FRAP, DPPH and ABTS assays), aerobic plate count, yeast and mold, and sensory evaluation of stored DGTD tea (0, 4, 8, 10 and 12 months) were evaluated.



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CHAPTER 2

LITERATURE REVIEWS

2.1 Rice

Rice (*Oryza sativa*) is a staple food for more than half of people in the world who consume rice as a daily food or as a main food. Around 95% of the world's rice is produced in developing countries especially in Asia. Rice is cultivated mostly in Asia and 90% are also consumed in Asia. Thailand is the sixth of the world that could produce rice (FAO, 2017). Rice is cultivated in Thailand throughout the country but the most popular area is Northeast of Thailand, which has 63.10% following by North, Middle and South of Thailand of 21.93%, 14.50 and 0.47%, respectively (Office of Agricultural Economics, 2018). Around 20 species of *Oryza* have been known. However, rice could grow in tropical and warm areas in both flooded land and highlands. Rice may have originally been cultivated in China (at least 7000 years ago), India (between 1500 and 2000 BC) and Indonesia (around 1648 BC). Thereafter, rice has been spread rapidly in southern and, southeastern Asia and other, tropical and subtropical regions, from about 2,300 years ago (Juliano, 1993).

Rice is a kind of cereal, which is very a good source of energy because rice contains various nutrients such as carbohydrate, protein and fat. Moreover, it contains fiber, vitamins and minerals to support and develop body growth. Brown rice, for example, consists of 7.3 g/100g protein, 2.2 g/100g fat, 71.1 g/100g carbohydrate, 0.29 mg/100g Thiamine, 0.8 mg/100g vitamin E, 3 mg/100g iron and 2 mg/100g zinc (Juliano, 1993). Rice grass was tested in the experiments described here and it consists of leaf (leaf sheaths and leaf blades), culm (stem, peduncle, elongated internode, node and unelongated internode), and roots (Figure 2.1) (Moldenhauer and Gibbons, 2003).



Figure 2.1: Morphology of rice grass: (A) leaf and culm, (B) whole culm

Rice has been classified in several ways including growth duration which can be divided into three phases that take at least 3 months but it can take 6-7 months in total. Rice could be classified by the kind of rice, which is divided into 6 categories (Thai Rice Foundation under Royal Patronage, 2006; Naivikul, 2007). Other terms of classification are as follows:

2.1.1 Classification of rice by origin

There are 2 kinds of rice consisting of African rice (*Oryza laberrima* Steud), which is cultivated in tropical African's and Asian rice (*Oryza sativa* L.), which is a hybrid of several wild rice types from India, Bangladesh and Southeast Asia. It is cultivated extensively in India as well as the golden triangle of Myanmar-Thailand-Laos and Vietnam and the South of China. Asian rice is further classified into 3 types (varieties);

a. *O. sativa* var. *Japonica* or *O. sativa* var. *Senica* is cultivated in the North and East of China, Japan, Korea and other countries in the warm zone. Its characteristics are plump; shorten seed and dark green leaves.

b. *O. sativa* var. *Indica* is cultivated in many countries in the tropical zone; including Sri Lanka, South and Middle of China, India, Indonesia, Bangladesh, Thailand and the Philippines. Its seed is tapering and its leaves are light green.

c. *O. sativa* var. *Javanica* is mostly found in Java in Indonesia and some in the Philippines, India and Sri Lanka. Its stem is strong, seeds have a tail and its leaves are light green.

2.1.2 Classification of rice by chemical composition of rice flesh

There are non-sticky rice and sticky rice, which are similar, except for the flesh of its husks. Non-sticky rice contains 15-30% amylose, while sticky rice contains 5-7% amylopectin.

2.1.3 Classification of rice by cultivated location

Upland rice is considered as local rice, cultivated for consumption as a staple food. Upland rice has many cultivars that can be divided into non-glutinous rice and glutinous rice or waxy rice. Non-glutinous rice varieties cultivars are listed as Jow Lisaw San-Pah-Tawng, Jow Haw, Goo Meuang Luang, Nang Kruan, Nang Kian, Sam Deuan, Leb Nok, Dawk Kam and Dawk Pa-Yawm. Glutinous rice or waxy rice varieties are such as Sew Mae Jan, Dam Ga Ton Dam and Ga Ton Keaw. Production of upland rice needs few water so it is cultivated in the highland or hill areas, normally without irrigation and often intercropped between trees (coconut, rubber tree or palm) after the old trees have been destroyed or before new cultivation. In Thailand upland areas are grown in the North, South, East and Northeast, which represent only about 10% of all rice cultivated area in Thailand. Upland rice in Thailand has been cultivated for many years in the North and South, which are only a small area in the East and Northeast.

Lowland rice is normally cultivated in swampy areas. Lowland rice needs to be grown in water until just before harvesting. The cultivated area in Thailand is located throughout the country constituting about 80% of all cultivated land, mainly in the Middle and Northeast. Lowland rice provides the average rice yield per rai more than Upland rice. The most commonly useful cultivars are Khao Dowk Mali 105, Pathumthani 1 and Suphanburi 60, for example.

Floating rice is cultivated by sowing dry seed during the early rainy season in uncontrolled water areas. The water is depths then rises to over 80 cm even to 3-4 m height, where the rice plants can adjust themselves to follow the water level. Floating rice is found in many Thai provinces (Ayutthaya, Suphanburi, Lop buri, Phichit, Angthong, Chainart and

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Singhburi, for example) in the Middle and some in the South. The cultivars include Loy or Khao Fang Loy, Pingaew 56, RD17, RD19, Nahng Cha-Lawn, Ta-Pow Gaew 161 and Leb Meu Nahng 111. The cultivated area is accounted for about 10% of all the cultivated area in Thailand and it provides the average rice yield of about 200-300 kg per rai.

2.1.4 Classification of rice by planting seasons

Normally Thai rice is only cultivated in the rainy season, normally from May to October, and harvested before February. There are photoperiod sensitive cultivars of upland rice, which is a direct mechanism for flowering, therefore the optimum growing season coincides with the shortest photoperiod in the early winter.

Off-season rice is normally cultivated in areas where irrigation is available. Day length does not affect blooming of lowland rice, since maturity depends solely on the age of rice, which is harvested around January to April.

2.1.5 Classification of rice by the duration time from seeding to harvesting

The time during cultivating from seeding to harvesting is the shortest for early cultivars which take only 90-100 days. Medium cultivars take 100-120 days and late cultivars take more than 120 days.

2.1.6 Classification of rice by shape (size) of grain

Size of grain can be classified into 4 ranges; short, medium, long and extra-long grain, which are less than or equal to 5.50, 5.51-6.60, 6.61-7.50 and over 7.51 mm, respectively.

2.2 Tea

Tea (*Camellia sinensis*) is a shrub that can grow up to 30 feet high, but is usually trimmed to 2-5 feet when cultivated for its leaves. The flowers are white and contain bright yellow stamens. These blossoms appear individually or as clusters. The fruits have hard green shells with round brown colored seeds (Willson and Clifford, 1992) (Figure 2.2).



Figure 2.2: Tea (*Camellia sinensis*)

Tea belongs to the family Theaceae and its botanical name is *Camellia sinensis* L. There are two main varieties, Chinese varieties or *C. sinensis* var. *sinensis* and Assamese varieties or *C. sinensis* var. *assamica*, which are mostly similar but different in origin, type of tea, their produces and physical characteristics. The Chinese variety is originated in China, growing in upland, cold areas and its leaves are small about 1×3 inches, erect narrow, serrate and dark green. (Figure 2.3(A)). The shrub is dwarf and slow growing. The Assam variety originated in India, growing in hot climate, it is tall and quick growing, its leaves are about 4×10 inches and are grown horizontally, are broad, mostly non-serrated and light green (Kitamura, 1950; Sealy, 1958). The characterize of tea are presented in Figure 2.3. (B)



Figure 2.3: *Camellia sinensis* varieties
(A) *C. sinensis* var. *sinensis*, (B) *C. sinensis* var. *assamica*

2.2.1 Tea harvesting

The simple act of plucking a leaf off a tea plant starts the chain of chemical reactions that leads to the various steps of tea processing. Harvested leaves are quickly brought to a nearby facility where tea production continues with withering. Fresh tea leaves are the most important for making high quality tea which is used only the first 2 leaves and bud with plucking by hand. The plucked tea is then put loosely in basket to avoid browning and breakages which may occur because of heat from the respiration of the tea leaves (Pongsupasamit, 1989). Chemically, the youngest leaves and buds contain the highest concentration of nutrients that reach their peak in early spring. Over 600 different flavors within the tea leaves have been identified, some of these can also act as insecticides in nature to repelling pests. These chemicals also responsible for the flavors and aromas in tea which consumers prefer. Plucking a tea leaves signals to the plant that it is being attacked; the plant's response is to release these compounds (Hampton, 1992).

2.2.2 Tea processing

Different processing methods can be used to make different teas i.e. green tea, black tea or oolong tea (Figure 2.4). For green tea, the leaves are steamed immediately after harvesting to inhibit oxidizing enzymes, particularly polyphenol oxidase, and withered to retain their fresh green color. For black tea and oolong tea, after plucking the leaves, they are withered for 5-20 h followed by fermentation by oxidizing enzymes. Black tea takes a longer processing time than oolong tea for the leaves to become brown, resulting the subsequently brewed tea being more pungent than non fermented tea or green tea. Therefore, oolong tea

is considered to be semi fermented tea and black tea is fermented tea (Surawatthanawan, 2007).

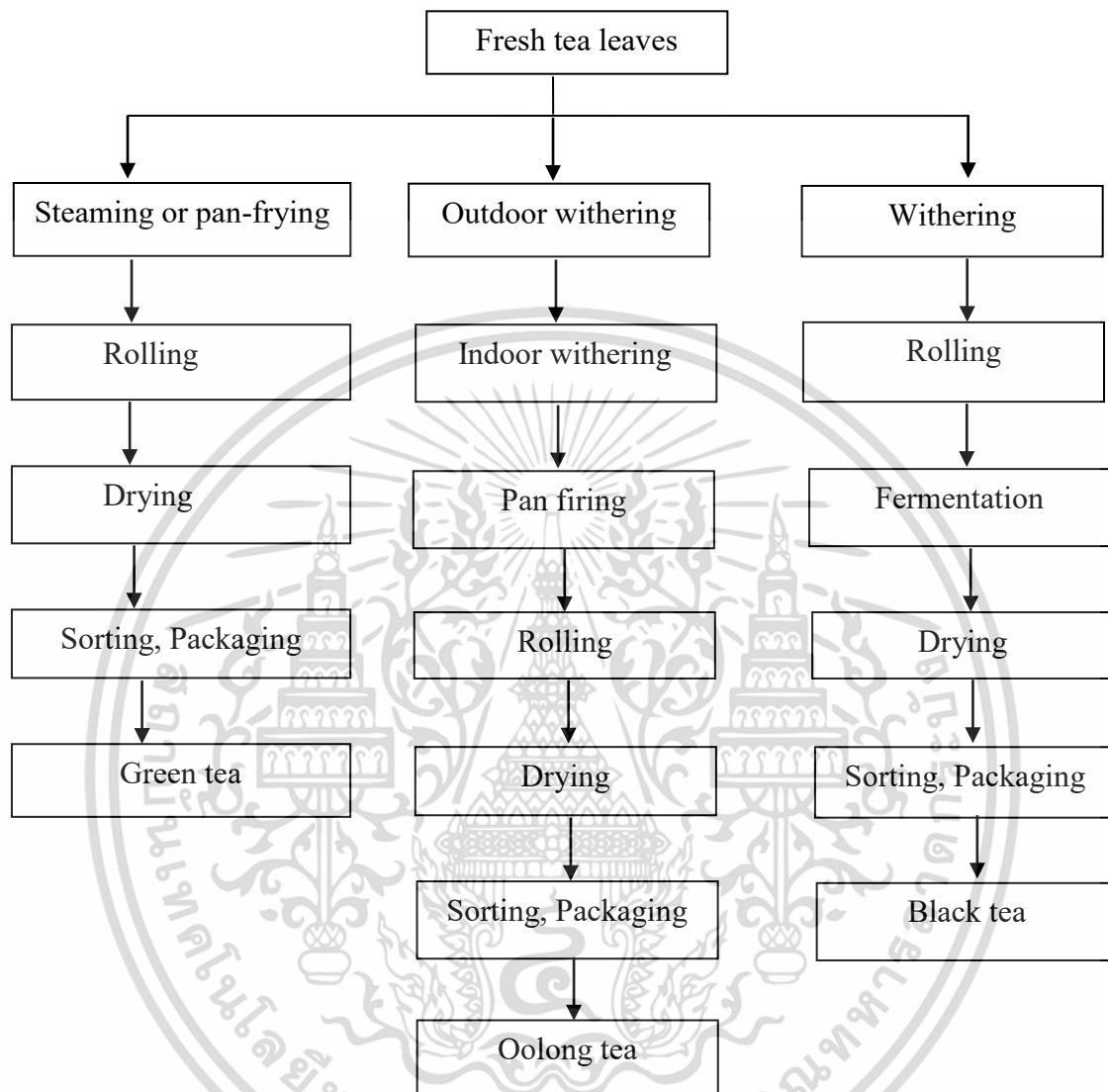


Figure 2.4: Processing of Tea

Source: Tea Institute Mea fah luang University, 2016

a. Withering

Withering step is the first step to reduce moisture in the leaves by spreading out on the floor or tray. The withering period of green tea is deliberately short, which is the reason for their green and vegetal taste.

Oolongs and black teas depend on the continued development of PPO to give them their eventual colors and aromas. Therefore, they have longer withering times. Because the เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า ไม่ว่าจะกรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ตัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้

layers of withering leave create heat, similar to compost accumulate, the leaves are continually turned during the process to dissolve heat and let more advancement of the flavors, color and aromas. Turning also imposes damage to the leaf structure which is pivotal for the upcoming step of oxidation. After withering, the tea leaves have a lower moisture level approximately 60-70%, but the leaves are still soft, flexible and prepare to be operated (Hampton, 1992).

b. Steaming/ Pan-frying

Tea leaves are spun during a hot metal cylinder where the leaves will be flushed with steam. This process is the most efficient in protecting the green color and vitamin level in the leaves. During pan frying the tea leaves are tossed around in a dry, hot metal wok by hand, but do not allow the leaves to burn. The purpose of steaming is to inactivate the enzymes by heat. Without steaming, enzyme in leaves change their components, which is oxidation of catechins caused by polyphenol oxidase. Catechins, are one of the main components of leaves, which are a factor in the astringent taste and are considered as having functions such as antioxidation. Steaming is important to keep the tea taste and to retain the functions of catechins in leaves onto tea (Hampton, 1992; Tokunaga, 2005).

c. Rolling

Rolling is normally operated by machine than by hand. Rolling the leaves until shaped into their final form or curl, but the leaf structure is increasingly damaged while rolling. Damage to the cells causes the liberate of polyphenols. Black and oolong teas, the polyphenols react with the yet active enzyme PPO and oxygen starts oxidation seriously (Tokunaga, 2005).

d. Oxidation/Fermentation

Oolong tea (semi-fermentation), black teas (fully fermentation) and green tea (non fermentation) have almost the same process except oxidation or fermentation in green tea since the enzyme PPO was deactivated. Oolong and black teas have high level of oxidation effect in flavor, color and aromas (Schapira, 1982; Hampton, 1992; Tokunaga, 2005).

e. Firing/ Drying

Drying step is intentional reduce moisture level in tea leaves which are without mold or decomposition after finish drying. Firing discloses tea leaves to heat from charcoal, tumble dryers, ovens, sun, steam or wood. Heating intensifies some of the tea flavors and

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commences the last of the chemical reactions to take place including the development of already supplementary aromatic compounds. The moisture content in the tea leaves has been decreased to proximately 3-6%. (Hampton, 1992; Tokunaga, 2005)

f. Sorting/Packing

Sorting is automatically process. Proper category is crucial for all grades of tea leaves in general. The sorting objective is to classify tea according to the size of the particles. In the past, this process was carried out by hand, with only instrument being a small tweezer used to pull out long stems. The process of sorting was known as screening at that time. There are several packaging methods used for tea. Teas require special protection against light, oxygen and moisture. Teas may be packaged as tea bags or loose tea. Normally for tea bags, 2 g of tea are packed into each tea bag while loose tea leaves are contained in metal foil aluminum bags or vacuum packed (Hampton, 1992).

2.2.3 Tea characteristics

a. Color of tea leaves

Dried tea is commonly dark brown or black. Chemical analysis showed that color of dried tea depends on chlorophyll in tea leaf which was destroyed in processing period, chlorophyll loses magnesium in acid conditions while in the fermentation process phaeophytin is produced which is black. Chlorophyll is also destroyed when chlorophyllase breaks down chlorophyll to phytol, which becomes chlorophyllide which is olive green. This activity occurs in the rolling period. In the drying process, chlorophyllide loses atoms and becomes phaeophorbide, which is brown in color. Therefore, the color of tea depends on ratio of phaeophytin and phaeophorbide. If phaeophytin is more than phaeophorbide, tea will be black, but if phaeophytin is less than phaeophorbide, tea will be brown (Pongsupasamit, 1989).

b. Color of tea

Color of tea depends on the fermentation period, it contains chlorophyllide from chlorophyll that is destroyed by enzyme action during processing. Tea could have many colors; yellow-green, yellow-brown or orange-brown. However, tea processed for a half fermented time will be light green (Pongsupasamit, 1989).

c. Aroma of tea

The aromas of tea are different depending on whether the leaves have been non-fermented half-fermented or fermented. Not only different processing of tea affects tea aroma but also different cultivars, weather and cultivated area. In a bio-chemical study E-2-hexanol and Z-3-hexanol from lipid of tea leaf were found. Linalool and geraniol were released from glucosides which were in tea leaves during withering. The fresh tea leaf aroma is less preserved in black and oolong than green tea because it is non-fermented. Tea aroma can be tested using gas chromatography which was shown that Chinese and Japanese green tea contain about 100 types of chemical aroma (Takeo, 1992).

2.2.4 Tea market in Thailand

Tea is one of the agricultural products commodities supported by the Government in Thailand. It is worth more than 8,000 million baht every year. The important cultivated areas are in Chiang rai, Chiang mai, Mae hong sorn, Prae and Nan. The Assam variety was the most cultivated at 87% with the Chinese variety was cultivated at 13%. Of this production 89% was used in Thailand and 11% was exported (Office of Agricultural Economics, 2015). In 2011-2015, the cultivated areas were increased from 126,340 rai to 135,437 rai or 1.80% per year. Tea production also increased from 73,320 tons to 81,127 tons or 2.71% per year. The production per rai also increased from 580 kg to 599 kg or 0.91% per year as presented in Table 2.1.

Table 2.1 Tea cultivated area in Thailand during 2011-2015

Year	Planting area (Rai)	Tea production (Ton)	Product/Rai (kg)
2011	126,340	73,320	580
2012	128,875	74,103	575
2013	132,229	77,090	583
2014	134,058	79,094	590
2015	135,437	81,127	599
Increasing (%)	1.8	2.71	0.91

Source: Office of Agricultural Economics, 2015

The cost of fresh tea production during 2011-2015 for Chinese tea increased from 21,139.99 baht/rai (2011) to 25,073.08 baht/rai (2015) or 5.82% per year. Assam tea increased from 3,936.74 baht/rai (2011) to 8,367.88 baht/rai (2015) or 19.91% per year because of the increasing of variable cost; wage, fertilizer and pesticide. Data was shown in Table 2.2. The price that farmers sold fresh tea leaves were Chinese oolong tea No.12 60-120 baht/kg, Chinese oolong tea No.17 120-200 baht/kg and Assam tea 10-20 baht/kg.

In term of export, tea leaves from Thailand increased from 1,212.70 tons (2011) to 1,839.91 tons (2015) or increasing at 9.92% per year. Green tea increased from 651.69 tons (2011) to 810.75 tons (2015) or 9.06% per year with rising value from 81.72 MB (2011) to 127.79 MB (2015) or 12.86% per year. Black tea increased from 562.01 tons (2011) to 1029.16 tons (2015) or 11.33% per year with rising value from 76.18 MB (2011) to 125.45 MB (2015) or 16.38% per year (Table 2.3).

Table 2.2 Statistics of production cost of fresh tea leaves in Thailand 2011-2015

Year	Type of tea	Variable cost (baht/rai)	Fixed cost (baht/rai)	Total cost (baht/rai)	Cost per unit (baht/kg)
2011	Chinese tea	18,586.09	2,553.90	21,139.99	28.53
	Assam tea	2,804.75	1,131.99	3,936.74	7.36
2012	Chinese tea	19,709.93	2,771.72	22,481.65	30.34
	Assam tea	3,149.37	1,200.60	4,349.97	8.13
2013	Chinese tea	21,345.34	2,943.44	24,288.78	31.13
	Assam tea	3,433.97	1,254.16	4,688.13	8.32
2014	Chinese tea	23,590.44	3,055.63	26,646.07	34.51
	Assam tea	4,621.19	1,294.05	5,915.24	6.16
2015	Chinese tea	22,955.52	2,806.45	25,761.97	33.89
	Assam tea	7,124.81	1,243.07	8,367.88	9.14
Increasing rate (%)	Chinese tea	6.20	2.90	5.82	4.87
	Assam tea	26.39	2.66	19.91	1.57

Source: Office of Agricultural Economics, 2015

Table 2.3 Quantity and value of exported tea leaves and tea products (2011-2015)

Year	Tea leaves					
	Green tea		Black tea		Total	
	Quantity (Ton)	Value Million (baht)	Quantity (Ton)	Value Million (baht)	Quantity (Tons)	Value Million (baht)
2011	651.69	81.72	562.01	76.18	1,212.70	157.90
2012	532.67	91.42	895.16	85.87	1,427.83	177.29
2013	998.15	119.37	584.34	92.66	1,582.49	212.03
2014	819.41	125.30	780.78	144.33	1,600.19	269.63
2015	810.75	127.79	1,029.16	125.45	1,839.91	253.24
Increasing rate (%)	9.06	12.86	11.33	16.38	9.92	14.61

Source: Office of Agricultural Economics, 2015

However, during this period, tea leaves were imported and increased from 3,056.71 tons (2011) to 6,988.94 tons (2015) or 11.51% per year. Green tea increased from 1213.56 tons (2011) to 3984.08 tons (2015) or 20.85% per year with rising value from 153.48 MB (2011) to 194.47 MB (2015) or 7.44% per year. Black tea increased from 1843.15 tons (2011) to 3004.86 tons (2015) or 3.85% per year with rising value from 150.06 MB (2011) to 272.95 MB (2015) or 15.60% per year as presented in Table 2.4.

Table 2.4 Quantity and value of imported tea leaves (2011-2015)

Year	Tea leaves					
	Green tea		Black tea		Total	
	Quantity (Ton)	Value (MB baht)	Quantity (Tons)	Value (MB baht)	Quantity (Tons)	Value (MB baht)
2011	1,213.56	153.48	1,843.15	150.06	3,056.71	303.54
2012	2,297.97	142.30	5,444.01	217.58	7,741.98	359.88
2013	1,733.13	150.92	2,988.39	223.07	4,721.52	373.99
2014	1,416.36	181.63	2,987.66	280.35	4,404.02	461.98
2015	3,984.08	194.47	3,004.86	272.95	6,988.94	467.42
Increasing rate (%)	20.85	7.44	3.85	15.60	11.51	11.77

Source: Office of Agricultural Economics, 2015

Chinese tea cultivars are popularly cultivated in the North of Thailand. There are many new tea cultivars that can be tested to combine these with other cultivars including Oolong tea no.12, Ching Ching oolong tea, Tei Guan Yin tea, Four season tea and Karn on oolong tea (soft stem tea) which is the most expensive due to their difficult cultivating methods and it is lower productivity. Generally, Assam cultivars and oolong tea no.12 cultivars are used for green tea production but green tea from oolong tea no.12 cultivars is more expensive than green tea from Assam cultivars due to its unique taste and odor (Department of Industrial Promotion, 2009).

Thailand mostly imported tea leaves from China, India, Indonesia and Japan. In 2007, Thailand imported 1,393 tons of tea with a valued of 108 million baht which was mainly from China, 591 tons valued at 21 million baht which was 42.4% of the total imported tea.

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2.2.5 Brewing tea

Brewing tea can be classified by the quality of tea which affect consumer acceptance. Perfect tea brewing has many factors including using fresh clear clean water, boiling, water temperature, time, teapot, quantity of tea and stirring. Brewing tea starts with the most proper tea weight, which is 2.5g/220ml of water to brew, put tea into the pot, after it has been preheated, then brew in 220ml water for 3 min. Green tea is best prepared using 75°C water for 2-3 min while oolong tea or floral tea 85°C for 2-3 min and 90-95°C water for 3-5 min is appropriate to make it as strong tea as black tea. Although brewing tea uses 100°C water tea can also be brewed at different temperatures along depending on the kind of tea. Tea consumption is required to be served at 65°C in a warmed cup with the proper brewing time to avoid over bitterness of tea and decrease the aesthetics (Pettigrew and Richardson, 2011; Dilmah School of Tea, 2017). The nutrition of brewed green tea was shown in Table 2.5.

Table 2.5 Nutritional value of dried brewed green tea

Nutritional Value per 100 g (3.5 oz)	
Energy	4 kJ (0.96 kcal)
Carbohydrates	0 g
Fat	0 g
Protein	0.2 g
Vitamins	
Thiamine (B ₁)	0.007 mg
Riboflavin (B ₂)	0.06 mg
Niacin (B ₃)	0.03 mg
Vitamin (B ₆)	0.005 mg
Vitamin C	0.3 mg
Minerals	
Calcium	0 mg
Iron	0.02 mg
Magnesium	1 mg

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Nutritional Value per 100 g (3.5 oz)

Manganese	0.18 mg
Potassium	8 mg
Sodium	1 mg
<i>Other Constituents</i>	
Water	99.9 mg
Caffeine	12 mg

Source: USDA Nutrition Report, 2013

a. Preparing of tea

The extraction of green, oolong and black tea leaves by brewing and using the right amount of tea to brew is critical, as is the method of brewing (Pettigrew and Richardson, 2011; Dilmah School of Tea, 2017).

b. Water

The tea beverage is 99% water, the good brew is come from a good water for being the good tea. Water which contains high amount of minerals or water which has been boiled many times is not proper for brewing tea. Contaminant water, chlorine or a defectively washed teapot can influence the consumer acceptance of a desirable tea. If the available water is full of mineral, or other chemicals, in particular calcium, it may be necessary to use a good activated carbon filter before brewing (Pettigrew and Richardson, 2011; Dilmah School of Tea, 2017).

c. Stirring

When the tea and hot boiled water are mixed, the leaves direct to the bottom of the teapot, decreasing the contact between boiled water and tea leaves. Stir the tea and let usual extraction. The optimum time to brew black tea is 3 min, oolong tea and green tea is 2 min, stirring tea again while adding water, and finally stir it again before serving (Dilmah School of Tea, 2017).

d. Water temperature and brewing time

There are many ways to brew tea with differing sizes of teapot. The most convenient and efficient way is pre-heat the teapot and tea cups by swirling about some hot water and

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draining it out before brewing. Due to green tea has a softer, mainly more gentle characteristics than black tea. Green tea is brewed properly in water at approximately 75°C for 2 min, whilst Oolong in water that is approximately 85°C for 2 min and black tea should be brewed at 90-95°C for 3 min (Pettigrew and Richardson, 2011; Dilmah School of Tea, 2017).

e. Consume condition

After brewing tea is finished, pouring tea into pre-heat teacup and to avoid over brewing, drained the tea out or took off tea leaves because over brewing tea developing bitterness. The optimum temperature of hot tea to serve is from acceptance of consumer which is around 65°C (Pettigrew and Richardson, 2011; Dilmah School of Tea, 2017).

2.3 Tea from other plants

Besides *Camellia sinensis* tea, other plant can also be used to make tea with the same processing as *C. sinensis* tea, for example from flowers, such as roses, chamomile, jasmine, chrysanthemum and leaves such as chamomile, *Morus alba*, mint, seeds such as rice and fruit such as cranberry, peach and blackberry etc, which have their own characteristics and health benefits such as reducing cholesterol. However, caffeine is absented in these mentioned teas. The consumption of this kind of tea is generally more for relaxation, health benefits, for example promoting energy and wellness, promoting a good night's sleep and also providing antioxidants to the body.

2.3.1 Herbal tea

Herbal teas or tisanes are commonly made from leaves, roots, seed or other parts of plants. They are favorite due to their aroma, flavor or health benefits of each herbal plant. They are produced by the same process as tea from *C. sinensis* (Ravikumar, 2014) and used heat with hot water to obtain herbal extraction. Herbal teas contain no caffeine, their taste is much appreciated and they are easy to drink either hot or cold. Most commercially available herbal tea contains 90% or more of herb tea and 10% of *C. sinensis* tea. For example, chamomile tea normally contains 90% of chamomile and 10% of tea but if it contains less than 10% of herbal tea, it is simply called tea.

A list of the common names of the plant or parts of the plant that are allowed to be used to make herbal tea by the Ministry of Public Health Thailand is shown in Table 2.6 and

quality or standard of herbal tea is shown in Table 2.7. ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
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Table 2.6 The common name list of plant or parts of plants used for making herbal tea

Common name	Scientific name
1. Bael Fruit	<i>Aegle marmelos</i> (L.) Corr.
2. Rosella	<i>Hibicus sabdariffa</i> (L.)
3. Ginger	<i>Zingiber officinale</i> Roscoe
4. Galangal	<i>Alpinia galanga</i> (L.) Willd
5. Lemon grass	<i>Cymbopogon citratus</i> (DC.) Stapf
6. White mulberry	<i>Morus alba</i> L.
7. Safflower (American saffron)	<i>Carthamus tinctorius</i> L.
8. Asiatic pennywort	<i>Centella asiatica</i> (L.) Urban
9. Pandanus	<i>Pandanus amaryllifolius</i> Roxb.
10. Chrysanthemum	<i>Chrysanthemum indicum</i> L
11. Lou Han Gua	<i>Momordica grosvenori</i> Swingle
12. Reishi (Ling Zhi)	<i>Ganoderma lucidum</i> (Fr.) Karst.
13. Indian gooseberry	<i>Phyllanthus emblica</i> L.
14. Jiaogulan	<i>Gynostemma pentaphyllum</i> (Thunb.) Mak.
15. Jewel vine	<i>Derris scandens</i> Benth.
16. Stevia	<i>Stevia rebaudiana</i> Bertoni

Source: Food and Drug Administration, 2006

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Table 2.7 Quality or standard of herbal tea

No.	List of qualities or standard	Regulation	Remark
1.	Moisture content	Regulations from Medication Act	In case of non-defined, less than 10% of weight is allowed
2.	Pathogenic microorganisms	Non detect <i>E.coli</i> in 1 g Non detect <i>Staphylococcus aureus</i> in 1 g Non detect <i>Clostridium spp.</i> in 10 g Non detect <i>Salmonella spp.</i> in 10 g	-
3.	Contaminant or toxic from microorganisms or other toxic substance ; - Arsenic - Cadmium - Lead - Copper - Zinc - Iron - Tin - Sulfur dioxide	Contaminated in safety amount for consumer; Less than 0.2 mg per 1 kg of food Less than 0.3 mg per 1 kg of food Less than 0.5 mg per 1 kg of food Less than 5 mg per 1 kg of food Less than 5 mg per 1 kg of food Less than 15 mg per 1 kg of food Less than 250 mg per 1 kg of food Less than 10 mg per 1 kg of food	Notification in herbal tea from the Ministry of Public Health No.98 B.E.2529 (1986) No. 280, 2003 No. 280, 2003 No. 280, 2003 No. 280, 2003 No. 280, 2003 No. 280, 2003 No. 280, 2003

Table 2.7 Quality or Standard of herbal tea (cont'd)

No.	List of qualities or standard	Regulation	Remark
- Mercury	- Aflatoxins	Less than 0.02 mg per 1 kg of food	No. 280, 2003
- Others	Pesticide	Less than 20 µg per 1 kg of food	No. 98, 1986
4.	Medicine or Psychotropic substances or drugs	Follow the regulations	No. 98, 1986
6.	Color	Contaminated in defined amount	Notification of the Ministry of Public Health No.288 B.E.2548 (2005)
7.	Flavoring from other objects	In case	-
		Not allowed	-
		Not allowed	Except flavoring from herb under defined from Ministry of Public Health in herbal tea

Source: Food and Drug Administration, 2006

2.3.1.1 Benefits of herbal tea

Herbal teas are made from different parts of plant (flower, leaf, fruit, branch, trunk, or root) and therefore they may consist of different properties including medicinal properties. Consumers usually know that herbal teas are normally consumed for therapy but herbal teas also provide relaxation and cleansing to the body. Different herbal teas have different uses and purposes and are dependent on the preferences of the consumers and each type of herbal plant are as follows: (Ravikumar, 2014)

a. Flower

Chamomile (*Chamaemelum nobile*) tea is the renowned herbal tea. It has a light soothing effect, using as a sleep assist. It also relieves stomach pains and a mild laxative. It helps relieve menstrual cramps.

Chrysanthemum (*Chrysanthemum* spp.) tea presents sweet taste. It can reduce body heat which arising from fever, helps shield liver and neutralizes toxins.

Red Clover (*Trifolium pretense*) tea can be used for inhibit menopausal symptoms, mastitis, cancer, asthma, bronchitis, psoriasis joint disorders and eczema. It is not advised for kids, pregnant women or breastfeeding women.

Safflower (*Carthamus tinctorius*) is not only beneficial in reducing cholesterol in blood arteries and reduces blood pressure but can also nourish the nervous system.

b. Leaf

Parsley assists kidney function and can be a diuretic. Peppermint is one of the best for releasing stress, helps digestive issues and refreshes up the breath, particularly when use it with chamomile.

Red raspberry tea is good for pregnant women or who are trying to become pregnant. It can be used to decrease labor pains and the uterus and pelvic muscles pains, but does not encourage compressions or miscarriage.

Stevia plant (*Stevia rebaudiana*) gives sweetness and able to use as sweetener instead of sugar

c. Fruit

Rose hips contain vitamin C and flavonoids which are a good treatment for fatigue, colds, and coughs.

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Cardamom tea helps digestion, prevents stomach pain and releases flatulence which is helpful for reducing dizziness, against pulmonary disease where plenty phlegm is and reduce coughs. Moreover cardamom tea relieves mood swings during menstrual period.

d. Bark from tree branches

Tea from cinnamon helps calm and assist healthy flowing and digestive system.

e. Roots

Ginseng tea encourages liveliness to keep the body stay healthy and warm. Ginger root tea is outstandingly for enhancing circulation, improving digestion, against nausea, lung congestion and inflammation of a joint. It is also good for nausea of any kind, from morning sickness to motion sickness.

2.3.2 Fruit tea

Fruit teas are famous beverages in hot water made from herbs, spices, fruits or other plant objects. Fruit tea is enhanced with the high level of bioactive compounds, antioxidants and vitamin C. Fruit teas have unique characters and interesting benefits to decrease total cholesterol, blood sugar and loss body's weight. Vitamin C provides to enhancing vaccinated system, decreases the infectious risk, conduces to controlling blood pressure and hypertension treatment (Futuremarketinsights, 2016).

Table 2.8 shows a ranking of the leading Twinings products in the United Kingdom from 2013 to 2017. In 2017, an estimated 5.6 million people consumed Twinings herbal, fruit or green tea, which was higher in 2013 to 2017 than any other types of Twinings tea in United Kingdom. Ranked second and third were Twinings' standard tea and other hot drinks.

Table 2.8 Twinings products ranked by number of consumers in the United Kingdom from 2013 to 2017 (in 1,000s)

Year	Twinings (herbal, fruit or green tea)	Twinings (standard or decaf tea)	Twinings (other hot drinks)	Twinings Iced Tea
2013	5,988	4,039	308	311
2014	5,840	4,340	308	366
2015	5,955	4,231	419	385
2016	5,601	4,101	328	331
2017	5,859	4,227	368	247

Source: Statista, 2017

2.4 Descriptive analysis

Descriptive testing is an analysis that is concerned with the sensory definition of products both qualitative and quantitative by trained panelists. Descriptive analysis is composed of 4 parts including quality characteristics analysis which use human senses to analyze the details of products such as appearance of the visual characteristics (color, texture, size). The second, intensity of quality characteristic analysis is an analysis that is concerned with category scales, line scales or magnitude estimation. Moreover, order of perception which is a sequence of perception or senses which are used to detect the sequence of sense during the test period. Lastly, overall impression is to continue all of perceptions and analyze them widely. (Chompreeda, 2007; Meilgaard et al., 1999; Stone and Sidel, 2004)

The algorithm of descriptive analysis

a. Subject selection

The panelists must be selected and well trained. They can be selected by using basic characteristics such as saltiness, sweetness or oiliness. Moreover, the panelists must also be able to perceive and classify odors. The panelists should quantify the sample and be able to give point score when they are served the test samples. For example, sucrose solution in different intensity (0, 0.15, 0.35, 0.55, 0.75, 1.00 and 1.50 %), the panelists who have acceptable perception system will be able to classify the samples sequentially and correctly. They will be tested many times with the same conditions or more difficult conditions until finally the panelists who pass the test will go on to test odors. The panelists who cannot pass the test will be rejected after this step. However, the panelist

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selection requires not only acceptable perception but also motivation and reliability, which are necessity for the profiling method. This method will take quite long time to develop. From the reasons above, the Quantitative Descriptive Analysis (QDA) test normally uses 10-20 trained panelists so in selection or screening at the initial number of persons should be at least 18-25 candidates (Chompreeda, 2007; Meilgaard *et al.*, 1999).

b. Attributes development

The next step is for the panelists to get used to the sample's characteristics by testing 3-10 samples each time, but taking care of their fatigued because fatigue can make the panelists less interested or focused on the test. The panelists endeavor to explain characteristics of samples and write their impressions down on paper without any discussion. Each panelist is in an individual booth. The panel leader then asks each panelist to report their findings to the other panelists and discuss the words or definitions. The panel leader is the person who manages, presents the samples to the panelists and reconciles any problem during the discussion. However, the panel leader will not guide or comment about creating the definitions for the panelists and the panel leader will not attend any test. This is to ensure that the panelists are free from the opinions and influence of the panel leader. The most relevant and suitable definitions will be selected from the discussions and form an agreement of the panelists that enable them to explain differences of each of the attributes and which are the important attributes of the samples. The definitions to be used for testing depend on the sample which will best explain the overall attributes for testing flavor attributes, aroma attributes, texture attributes, appearance attributes or only one of these. The definition should be a single word and be clear, such as "creaminess" should be divided into smoothness, viscosity, fatty mouth feel and cream flavor. The panel leader may distribute the standard of each attributes to assist the panelists to describe and come to an agreement (Chompreeda, 2007; Meilgaard *et al.*, 1999).

c. Scaling technique

This part of the panelists training about scaling and practicing. An unstructured line scale is used, which is 15cm long and divided into 1cm intervals from head to tail. There is only min and max values on the head and tail of line scale and only bipolar words are used. The panelists give points for each attribute by marking on the line where they decide the attribute should be. This scaling technique is popular, especially descriptive analysis, which does not use category scales or numerical scales (these are hedonic scales that

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commonly use, for example most liked = 9, most disliked = 1) because this has been shown to reduce the panelists bias (Chompreeda, 2007; Meilgaard *et al.*, 1999).

d. Samples evaluation

The panelists must test and evaluate all the attributes which have been agreed. Each panelist will be in a separate booth and will make their assessments without any discussion or consultation while testing. For good management standards of evaluation many factors must be taken into account including using random digits as the identifier of the sample, the booth lighting must be appropriate and drinking water for clearing and cleaning mouth between testing samples must be used. In some cases, bread or plain crackers need to be supplied for the panelist to eat between samples. Each sample must be tested and evaluated twice or three times for consistency of each panelists and the panel leader should be confident that the panelists can classify each product's attributes, or some of attributes. If the panel leader is not confident the panelist must practice more. The results of the evaluation will be converted into numerical values, always measure from the head, and then statistical analysis will be carried out of the numerical data (Chompreeda, 2007; Meilgaard *et al.*, 1999).

2.5 Reviewed of relevant papers

2.5.1 Product from rice leaves

The attempted on utilizing rice leaves from several rice cultivars to produce health tea or juice has been reviewed as follows;

Sudtasarn *et al.* (2008) used fragrance rice seedlings (Kao Dawk Mali 105 (KDML 105), Pathumthani 1 and Sakonnakorn) to produce green tea and found that the most suitable age of seedling was between 14-21 days old. They used pan frying and dehydrated at 80°C as the process to achieve a moisture content of 5.50-6.05%. The characteristics of green tea products from all three cultivars were similar, in terms of color and taste. However, aroma of green tea from KDML 105 was better than the others. The nutritional properties of green tea product included calcium 365.40-422.30 mg/100g (maximum in Sakonnakorn), sodium 20.40-31.60 mg/100g (maximum in Sakonnakorn), magnesium 194.20-421.40 mg/100g (maximum in Sakonnakorn), potassium 1180.60-1925.20 mg/100g (maximum in Pathumthani 1) iron 6.06-11.00 mg/100g (maximum in Sakonnakorn). Vitamin B1 0.49-0.55 mg/100g (maximum in Sakonnakorn), Vitamin B2 0.70-0.90 mg/100g (maximum in KDML 105), Vitamin C 4.42-6.60 mg/100g (maximum in Sakonnakorn), Vitamin E 4.18-

5.34 mg/100g (maximum in KDML 105), chlorophyll 7.68-8.69 mg/100g (maximum in KDML 105) and β -glucan 4.01-4.16 mg/100g (maximum in KDML 105).

Khanthapok (2015) studied juice squeezed from seven colors rice grasses (Kum Doisaket, Kum Ka, Kum Noi, Kum Pe, Kum Ton Khieaw, Niaw Dum Chor Mai Phi and Rice berry) and seven white rice grasses (Khai Mod Rin 3, Khao Dawk Mali 105, Khao Gaw Diaw 35, Leb Nok Pattani, Pathum Thani 1, Plai Ngahm Prachin Buri and RD6) compared with Fang 60 as wheatgrass (*Triticum aestivum*). The antioxidant activities were determined using total phenolic content, antioxidant activities by DPPH and FRAP assays, β -carotene, total monomeric anthocyanin content, thiobarbituric acid reactive substances assays and evaluation of the assurance against oxidative DNA damage. The results showed the colored of rice grass juices presented more antioxidant activity than the white rice grass juices and wheat. The colored rice grass juice cultivar Kum Doisaket presented the greatest antioxidant capacity in all assays. The colored rice cultivars Kum Doisaket and Kum Noi and wheat were also investigated about the DNA shielding properties and only the rice grass juice of Kum Doisaket cultivar showed a dose-dependent DNA protective effect. The remarkable antioxidant potency for the Kum Doisaket cultivar may be influenced by the high amount of anthocyanin show in its rice grass juice. From the study, it showed the occasion of developing practical foods product from colored rice grass.

Yun (2016) determined antioxidant activities, total phenolic content, total chlorophyll and other physical properties including pH, yield and color of squeezed rice grass juice, prepared from Leb Nok-LN (white non waxy rice) and Ka Ton Dam-KTD (black waxy rice), which were Thai upland rice cultivars, KTD was selected at 4 weeks of the age for freeze drying process because of the preferable aroma, the maximum of antioxidant activities, bioactive compounds and high yield of rice grass. Antioxidant activities (DPPH: 23.51, ABTS: 44.56, FRAP: 34.58 mM TE/ml) and TPC (TPC: 1.98 mg GE/ml) of LN increased and maximized on week 2. Antioxidant activities (DPPH: 9.09-19.86, ABTS: 24.63-37.40, FRAP: 13.91-24.11 mM TE/ml) and TPC (1.03-1.72 mg GE/ml) of KTD increased by growth ages. Nevertheless, antioxidant activities (DPPH: 10.66, ABTS: 31.24, FRAP: 16.40 mM TE/ml) and TPC (1.11 mg GE/ml) of wheatgrass were lower than upland rice grasses. Total chlorophyll of LN (0.16-0.26 mg/ml) and KTD (0.13-0.18 mg/ml) cultivars, increased by age, however they were lower than of the wheatgrass (0.30 mg/ml). The 4-week-old KTD rice grass juice contained high vitamins: B5 (2.67 mg/100g), B3 (0.85 mg/100g) and E (0.34 mg/100g) and also minerals: potassium (542.00

mg/100g), magnesium (66.80 mg/100g) and phosphorus (54.00 mg/100g). The freeze-dried powder of KTD with 8% maltodextrin had low water activity (0.23) and low moisture content (5.98%). Antioxidant activities, TPC and color of reconstituted juice of KTD freeze dried powder were a little change for 4 months storage. From the study, LN and KTD upland rice grass juice contain good antioxidants, phenolic content and chlorophyll which could provide health benefits. Freeze drying process is a suitable drying method for KTD juice to prevent the loss of antioxidant activities, phenolic content and chlorophyll.

2.5.2 Tea from other plants

As indicated earlier that teas have been made from various plant species, some have been tested that were not acceptable but many have proved acceptable and beneficial and are marketed commercially throughout the world. The following researches are some of the reviews of these products,

Banchobphutsa and Rinthara (2012) researched for result of *Morus alba* leaf tea to dyslipidemia persons. Experimental, involved 46 persons which were divided in to two groups, the first group received *Morus alba* leaf tea 2 grams per day, 3 times a day for 8 weeks and another group (control) did not receive *Morus alba* leaf tea. The result showed that the group that drank *Morus alba* leaf tea had significantly ($p \leq 0.05$) different blood test results for total cholesterol, triglyceride, LDL-C (low density lipoprotein) and fasting blood sugar. But HDL-C (high density lipoprotein) was reduced. Total cholesterol reduced from 230.22 to 207.65 (9.8%), triglyceride reduced from 120.78 to 102.7 (14.9%), LDL-C reduced from 131.57 to 128.84 (2.02%), fasting blood sugar (FBS) reduced from 97.65 to 85.43 (12.5%). But the control group (did not drink) were not different in total cholesterol, triglyceride, LDL-C and HDL-C but only FBS was significantly reduced.

Sahin (2013) determined total phenol, total flavonoid, and total anthocyanin levels in 16 several fruit teas made in Turkey. The antioxidant properties of fruit teas between 0.96 to 6.91 mg GAE/g dried fruit tea. Pomegranate had significanty high total phenol content brewing at 100°C. The total flavonoid content was evaluated by using the UV-Visible spectrophotometric and varied from 1.70 to 36.81 mg TE/g dried fruit tea, peach had the highest level of total flavonoid content among other fruit teas brewing at 100°C. Total anthocyanin content in fruit tea infusions was also determined by using the UV-Visible

spectrophotometric and found to be higher when brewed at 100°C, and blackberry contained the highest anthocyanin.

Limsettho (2014) determined TPC and radical scavenging activities by DPPH and ABTS assays of tea leaves (*Camellia sinensis L.*), tea mulberry leaves and tea moringa leaves were extracted by 100°C water for 2, 5, 10, 30 and 60 min. The result found that tea leaves extract had the maximum of TPC at 2 min was 80.45 µg GAE/ml and the maximum of radical scavenging activity DPPH and ABTS for 60 min were 88.58% and 98.00%, respectively. The highest TPC of moringa and mulberry tea leaves extracted by hot water for 60 min were 43.71 and 34.34µg GAE/ml, respectively. Radical scavenging activities DPPH and ABTS at 2 min of tea moringa leaves extract were 87.98% and 98.22%, respectively, and of tea mulberry leaves extract were 88.41% and 98.01%, respectively. It may be concluded that tea from mulberry leaves and moringa leaves had radical scavenging activities in shorter extracting time than tea leaves (*Camellia sinensis L.*).

Nantachai *et al.* (2014) determined the quality of pomelo peel tea by the processing of by-product from pomelo peel cultivar Khao Nam Pung. The result showed that all conditions (Rolling for 10 and 20 min, fermentation for 1 and 3 h and drying at 50°C and 60°C) were not different significantly ($p>0.05$) in phenolic contents which was 123.79 – 211.88 µg gallic/ml. The antioxidant activities by DPPH assay of the sample fermented for 1 h was significantly higher ($p<0.05$) than 3 h in the range of 61.74-62.15%. However, rolling time and drying temperature did not affect antioxidant activities. The most acceptances from sensory evaluation were process condition of rolling for 20 min, fermented for 1 h and drying at 50°C. The preference scores by 9-points hedonic scale of color, odor, taste and overall acceptance were 7.20, 6.77, 6.43 and 6.97, respectively.

Othong *et al.* (2015) compared antioxidant activities and TPC of yanang (*Tiliacora triandra*) juice with yanang tea, yanang mixed with chrysanthemum tea and pandan (*Pandanus amaryllifolius*) leaf tea. The result was found that antioxidant activities by FRAP and DPPH, and total phenolic compounds in yanang tea, yanang mixed with chrysanthemum tea and with pandan leaves tea contained more than yanang juice. In the range of FRAP and DPPH, and total phenolic compound were 0.50, 1.45 mgTE/100 mL and 0.63 mg GAE/100 mL were in yanang juice respectively, whereas the range of FRAP and DPPH, and total phenolic compound were 2.30, 6.34 mgTE/100 mL and 0.72 mg GAE/100 mL were in yanang tea respectively, 3.11, 6.60 mgTE/100 mL and 1.39 mg

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GAE/100 mL were in yanang mixed with chrysanthemum tea respectively and 2.48, 6.46 mgTE/100 mL and 1.05 mg GAE/100 mL were in pandan leaves tea respectively. Therefore, the yanang mixed with chrysanthemum tea contained the highest amount of antioxidant capacities and TPC compared with the others.

Sinthupibulyakit *et al.* (2015) determined TPC and the antioxidant capacities of ready-to-drink herbal teas which were *Camellia sinensis* (control), bael tea, roselle tea, chrysanthemum tea, and lemongrass tea. The bael tea (*Aegle marmelos*), was the highest in comparing with roselle tea (*Hibiscus sabdariffa*), chrysanthemum tea (*Chrysanthemum sp.*), and lemongrass tea (*Cymbopogon citratus*) significantly in the TPC and the antioxidant capacity. Lemongrass tea contained the least in TPC and the antioxidant activity compared with tea from *Camellia sinensis* (control) and the other herbal teas. The correlation coefficient analysis of the TPC and the antioxidant capacities of 3 the herbal teas, was found that bael tea, roselle tea, and lemongrass tea were got positive correlation coefficient of the TPC and the antioxidant activities, the TPC which was the main antioxidant activities to inhibit free radicals. On the other hand, the negative correlation coefficient analysis of the total phenolic compounds and the antioxidant activities of chrysanthemum tea showed that the TPC may not be the main antioxidant to inhibit free radicals.

2.5.3 Antioxidant activities in green tea

Pereira *et al.* (2014) studied investigation of TPC, flavonoid and antioxidant capacity of green tea, black tea and white tea of *Camellia sinensis* (L.). The study was attended to compare the TPC, flavonoid and antioxidant activity of green tea, black tea and white tea bags of different brands. The results showed the variation in both TPC from 30.55 to 60.85 mg of pyrogallol/g and flavonoids from 6.35 to 8.92 mg of quercetin/g. Green and white teas were found to have the highest antioxidant activities as measured by ABTS and DPPH.

Lee *et al.* (2014) determined the relationship between the plucking periods which were very young leaf or first flush leaf, second flush leaf, third flush leaf, and old leaf or fourth flush leaf and the major constituents and the antioxidant capacity in green tea. The result showed the amounts of theanine, theobromine, caffeine, catechin (C), and gallic catechin gallate (GCg) were significantly reduced, while epicatechin (EC), epigallocatechin gallate (EGCg) and epigallocatechin (EGC) were significantly increased with the plucking period.

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Moreover, antioxidant capacity of green tea and standard catechins was determined using ABTS, FRAP and DPPH assays. The greatest antioxidant capacity was noticed in comparing the oldest leaf, irrespectively of the methods used. Moreover, the sequence of antioxidant activity of standard catechins was as follows: EGCg \geq GCg \geq ECg $>$ EGC \geq GC \geq EC \geq C. Additionally, the cis-catechins levels were the key constituent pathetic the antioxidant capacity of green tea in all assays operated (ABTS, which had $r = 0.731$, $p < 0.01$ and; FRAP, $r = 0.886$, $p < 0.01$ and; DPPH, $r = 0.778$, $p < 0.01$).

Shannon *et al.* (2018) studied comparison of the TPC, antioxidant capacity by FRAP, DPPH, total flavonoid content (TFC), and caffeine level of five teas (black tea, green tea, white tea, chamomile tea and mixed berry/hibiscus tea) from local supermarkets in Ireland. The brewing times were within 0.5-10 min at 90°C. The result showed that green then black teas had the greatest of all contents for 5 min brewed. TPC were 557.58 and 499.19 μg GAE/g, TFC were 367.84 and 325.18 μg QE/g, FRAP were 887.38 and 209.38 μg TE/g, and DPPH radical scavenging capacity were 1233.03 and 866.39 μg AAE/g. Caffeine level per serving (200 mL) in black tea, green tea, and white tea were 63, 51, and 49 mg, respectively. However chamomile and mixed berry tea had zero caffeine detected. Moreover, comparison between five teas (green, black, white, chamomile, and berry tea) and orange juice and apple juice, the result found that TPC and DPPH radical scavenging capacity in five teas were higher than orange juice and apple juice. The conclusion showed that green and black teas were significant sources of antioxidant activities and TPC.

2.5.4 Brewing tea

Labbe *et al.* (2006) studied on using electromigration technology to demonstrate intensity of EGC and EGCG of green tea. Effects of temperature at 50, 60, 70, 80 and 90°C and brewing time for 0, 5, 10, 20, 40 and 80 min on the catechin solubility from green tea were studied. Brewing temperature and time affected on catechin and caffeine intensity. Catechin was able to divide into 2 groups as the time dependent compounds (EGC and EC) and the time and temperature dependent compounds (C, EGCG, GCG and ECG). The optimal extraction time and temperature was 50°C for 20 to 40 min for time dependent compounds, 90°C for 80 min for the time and temperature dependent compounds, and 70 to 80°C for 20 to 40 min for caffeine content.

Kitpot *et al.* (2007) studied color quality and consumer acceptance affected by temperature and time of brewing green tea and Jiaogulan tea (*Gynostemma pentaphyllum*). The conditions were brewing in 100 mL water at 60, 80, 100°C for 1, 5 and 10 min. The 32 factorial designs were used for this experimental. The consumer acceptance test was conducted by using hedonic scaling (n=40) and the color measurement in L^* , a^* , b^* and ΔE^* . The most proper green tea immersion was brewing in 100°C for 1 min that the average rating of overall liking, color, odor, flavor and aftertaste were 5.24, 5.26, 5.98, 5.48 and 5.50, respectively. The color of optimum green tea infusion in L^* , a^* , b^* and ΔE^* were 83.56, -3.56, 48.89 and 51.26, respectively. The suitable condition of Jiaogulan tea that the average rating of overall liking, color, odor, flavor, and aftertaste were 5.72, 5.03, 5.13, 5.58 and 5.63 respectively. The color of optimum Jiaogulan tea infusion in L^* , a^* , b^* and ΔE^* were 90.96, -6.06, 22.85 and 24.58, respectively which was brewing in 100°C for 1 min as well.

Saklar *et al.* (2015) studied effects of brewing conditions of Turkish green tea on catechins and sensory characteristics. The samples were prepared in several ways at 75, 85 or 95°C for 1, 2, 3, 5, 10, 20, 30 or 45 min. They were then analyzed for the amounts of epistructured catechins (EGCG, EGC, ECG, EC), non-epistructured catechins (C, GC, GCG) and caffeine in the brewed tea samples. The result showed that the most suitable brewing condition was 85°C for 3 min. Sensory characteristics were analyzed by nine trained panelists and this also showed that 85°C for 3 min were the optimal conditions. There was a rapid increase in epistructured catechins for the first 3-5 min of brewing at 85°C, but an increased brewing time resulted in a decrease in the yield of epistructured catechins and the amount of non-epistructured catechins but this was increased continuously with longer extraction times. Additionally, sensory scores for color, taste, aroma and overall acceptance were highest at 3 and 5 min brewing times at all temperatures, however the scores were very low for 30 and 45 min of brewing at 85 and 95°C because there was a bitter taste and a dark color.

2.5.5 Shelf life of green tea

Siripatrawan *et al.* (2009) investigated methods that could be used to prolong shelf life of mulberry leaf tea (*Morus alba*) packed in different packaging materials and storage conditions. The mulberry leaf tea was packaged in polypropylene bag under atmospheric air (PP), nylon bag under atmospheric air (PA), nylon bag under vacuum condition (V-PA), aluminum bag under atmospheric air (AL) and aluminum bag under vacuum

condition (V-AL) and stored at 30°C and 70% RH. Water activity, colors (L^* , a^* , b^* values and ΔE^*), total phenolic compounds, total flavonoids, sensory evaluation (color and odor) and infused tea (color, odor and flavor) were evaluated at 30 days interval. The results showed that packaging materials significantly affected ($p < 0.05$) water activity, colors, total phenolic compounds, and total flavonoids of the product. The products were also found that mulberry leaf tea packaged in PP, PA, V-PA, and AL had shelf life of 5, 7, 7 and 10 months, respectively, while tea packaged in V-AL could be stored for up to 12 months.

Lee and Chamber (2010) determined flavor changing in green teas that are stored over a period of 2 years (a commonly noted shelf life for green tea products in loose leaf form). Two Korean green teas were packed in metalized multilayer polyethylene films and stored at 20°C. Then the samples, which were 3, 6, 12, 18 and 24 months after their original packaging dates, were evaluated by six highly trained descriptive panelists. The green tea samples that were stored for 6 months did not change, up to 12 months they changed little, and after 12 months the samples sometimes developed low levels of off-flavors. In overall, the findings of this study indicated that green tea would change minimally during the first year of storage and would change slightly more during the second years of storage. However, these changes appeared to be minimal when the samples were packaged in metalized multilayer polyethylene films.

Nualbunruang *et al.* (2014) studied on improvement odor and flavor in tea by adding white Champaka. Dried oolong tea mixed with dried white Champaka petal at the ratio of 1:1 was used to prepare tea at different fermentation temperatures (30 and 40°C) and times (5 and 15 min). Fermentation step at ambient temperature for 15 min was the control treatment. The results revealed that the linalool contents in the mixed tea were in the range of 0.4 - 2.0 mg/ kg. The tea made from 5 methods was evaluated for organoleptic analysis using 30 trained panelists. The tea mixed with white Champaka got scores at the level of slightly to moderate like for the quality attributes of color, odor and white Champaka odor ($p < 0.05$). The scores of flavor, after taste and overall preference were not significantly different ($p \geq 0.05$). White Champaka mixed tea in sealed plastic bag was kept at ambient temperature for 1 and 2 months. It was found that increasing storage time expressed higher contents of water activity (A_w) but lower the red (a) and yellow (b) color values ($p < 0.05$) in the tea while the lightness values were not influenced by storage time. The tea product had the yeast and mold counts less than 3.6 log cfu/g after 2-month storage.

เอกรินทร์ วัฒนศิริกุล (2014) ศึกษาการปรับปรุงกลิ่นและรสชาติชาอู่หลงโดยผสมดอก Champaka ขาว

ไม่ว่ากรรมใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้คัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้

2.5.6 Sensory characteristics of green tea

Lee and Chambers (2007) studied on a lexicon for descriptive analysis of green tea by 6 highly trained panels which had completed 120 h of general training and had minimum 1200 h for general sensory testing including beverages, vegetables and other fruit products with descriptors similar to those might be found in tea. One-hundred and thirty-eight green tea samples from various countries origin (China, India, Japan, Kenya, Korea, Sri Lanka, Taiwan, Tanzania and Vietnam) were selected to represent green teas. Six grams of green tea leaves was placed in teapot followed by 300 mL of 70°C water brewed it for 2 min with swirling 10 times. The result showed that 6 highly trained panels identified, defined and referenced 31 flavor attributes for green tea by descriptive analysis. The attributes could be classified into 6 groups as green, brown, fruity/floral, mouthfeel, basic tastes, and other attributes. There were “Green” (asparagus, beany, Brussels sprout, celery, parsley, spinach, green beans, green herb-like); “Brown” (ashy/sooty, brown spice, burnt/scorched, nutty, tobacco); “Fruity/Floral” (fruity, floral/perfumy, citrus, fermented); “Mouthfeel” (astringent, tooth-etching); “Basic Tastes” (overall sweet, bitter); and other attributes (almond, animalic, grain, musty/new leather, mint, seaweed, straw-like). Some attributes, such as green, brown, bitter, astringent and tooth-etching, were found in most samples, but many attributes were found in only a few samples.

Vittayaporn and Chompreeda (2010) evaluated 7 commercial green tea with roasted Hom-mali brown rice samples using eight trained panelists and identified 18 attributes. Seven samples consisted of 3 brands of dried leaf tea and 4 brands of tea bags. It was found that consumer preferred tea bags than dried leaf tea and the preference mapping identified attributes into 2 PCs (Principal Component); with PC1 was the aroma and flavor of green tea that could describe 66.80% of the variation and PC2 was the taste and aftertaste of tea that explained 17.10% of the variation. The optimum process of making green tea added with roasted brown rice was cooking Hom-mali brown rice (500 g) for 12 min, tray drying at 70°C for 100 min and roasting at 200°C for 5 min. The optimum ratio of green tea: roasted Hom-mali brown rice was 1.0:1.1. Green tea with roasted Hom-mali brown rice had a 2.33% of moisture content. The higher tea and dry aromas, but the less intense roast aftertaste was the attributes that may drive consumers’ liking of green tea with roasted brown rice. For acceptability test by using 200 consumers, preferred was attained moderately.

Leaves of the Rang Chuet (*Thunbergia laurifolia*) tree are a traditional Thai medicine. Chiang *et al.* (2011) made comparisons with the commercial Rang Chuet (RC). Four different drying methods (microwave-dried (MD), freeze-dried (FD), oven-dried (OD) and freeze-withered (FW)) were evaluated by using quantitative descriptive analysis (QDA) by 9 trained panels and consumer acceptability based on a 9 point hedonic scale by using 129 untrained panels. The result showed quantitative descriptive analysis of *Thunbergia Laurifolia* teas produced from different drying methods were 8 attributes; fermented, unfermented, bitterness, astringency, glassiness and sourness. MD and FD teas could be classified as green tea (unfermented), while OD, RC and FW teas were characteristic of black teas (fermented). Results of the hedonic survey, which scored attributes of appearance, aroma, flavor, overall acceptability and preference ranking, showed that the MD tea was the most preferred, being the least bitter and has a sweet after taste. The OD tea had the lowest scores for aroma, flavor and overall acceptability.

2.5.7 Gas chromatography using headspace solid phase microextraction in teas

SPME was developed in the 1990s by (Arthur and Pawliszyn, 1990) and every year a thousand papers describing different aspects of this approach, and applications in different fields (chemical analysis, bioanalysis, food science, environmental science, and recently, pharmaceutical and medical sciences), are published (Bojko *et al.*, 2012). This sample extraction technique was demonstrated to be rapid, simple, and reproducible, with no solvent use, and is suitable for the extraction and concentration of a high number of volatile and semi-volatile compounds from aqueous solutions (Harmon, 1997; Yu *et al.*, 2012). Moreover, SPME needs a small sample volume and the coupling with gas chromatography and mass spectrometry (GC/MS) provides high sensitivity. For these reasons it has been used to study the volatile profile of many type of fruit, vegetables, and beverages, including tea, grapes and wine (Vas and Vékey, 2004; Castro *et al.*, 2008; Flamini, 2010).

Lv *et al.* (2012) studied aroma characterization of Pu-erh tea using headspace-solid phase microextraction by GC-MS. 28 Pu-erh tea samples and 14 Pu-erh raw tea samples were provided by Biological Resource Development and Innovation Office of the People's Government of Yunnan Province, which were subjected to sensory evaluation by 3 experts then one Pu-erh tea was selected to be compared with green tea, black tea, oolong tea and

white tea. Ten grams of each tea samples, previously homogenized, were put into a 150

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mL vial and filled with 30 mL boiling water then immediately sealed and kept at 60°C for 5 min to equilibrate in water bath then SPME fiber was exposed for 60 min after that introduced it into the GC injector (250°C). The oven was programmed at 50°C for 5 min then increased to 125°C at a rate of 3°C/min, the held for 3 min and to 180°C at a rate 2°C/min held for 3 min and last to 230°C at a rate of 15°C/min. For mass spectrometer conditions: ionization mode, EI; electron energy, 70 eV; interface temperature, 280°C; ion source temperature 230°C; quadrupole temperature, 150°C, mass can range, 35-400 AMU; emission current, 34.6 μ A. The internal standard, which was analyzed by using ethyl decylate instead, showed that its peak area was poor in repeatability and stability, were not used in this study. The result showed GC-MS chromatography of the flavor profile of Pu-erh tea identified 66 major volatile compounds including 9 methoxy-phenolic compounds, 13 alcohols, 9 aldehydes, 9 ketones, 4 esters, 2 lactones, 17 hydrocarbons, 1 acid and 2 phenolic compounds. The two-sample t-test showed that Pu-erh tea was significantly higher in the methoxy-phenolic compounds compared to green tea, black tea, oolong tea and white tea. Thus this characteristic aroma in Pu-erh can be recognizing from other teas. In addition the content of 3 major compounds, 1,2,4-trimethoxybenzene, 1,2-dimethoxy benzene and 1,2,4-trimethoxy benzene in Pu-erh raw tea were 2.18%, 1.31% and 0.44% respectively. Thus it can be show that that post-fermentative process had radically adjusted the profile of the Pu-erh tea.

Beldermann *et al.* (2014) was able to discriminate between green, oolong and black teas by GC-MS analysis of characteristic volatile flavor compounds. They tested 38 tea products from China, Japan, Indonesia, Sri-Lanka and Taipei which consisted of 7 green teas (non-fermented tea), 13 oolong teas (semi-fermented tea) and 18 black teas (fully fermented tea). The products were analyzed by GC-MS to identify each tea. 2 g of tea product was added to 40 mL of water at, 80°C and allowed to brew for 5 min then the supernatant was removed by centrifugation at 3000 g for 10 min then analyzed. One μ L concentrate was subjected to GC-MS for identifying and relative quantification of volatiles. The results showed that the relative peak areas determined for 82 volatiles were hexanal, undecan, methylpentenone derivative, 1-penten-3-ol, unknown 1, methylbutanol derivative, 2-hexenal,(E)-, 2,5-dimethylpyrazin, diethyl benzene, 2,5-dimethyl pyrazine, 2-penten-1-ol, benzene,1,3-diethyl-, 2-ethyl pyrazine, 1-hexanol, 3-hexen-1-ol, 2-hexen-1-ol, (E)-, pentylalcohol, linalool oxide I, 2-furancarboxaldehyde, 2,4-heptadienal, furan,2,5-dimethyl, linalool oxide II, furan-2-propyl, ethanone, 1-(2-furanyl)-, benzaldehyde,

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Linalool, 2-furancarboxaldehyde, 5-methyl-, 2-heptanone, 5-methyl-, 1H-pyrrole-2-carboxaldehyde, 1,5,7-octatrien-3-ol, 3,7-dimethyl, benzenamine, 2-methoxy-5-methyl, 2-furanmethanol, 2(3H)-furanone, 5-ethyl-dihydro, 2(3H)-furanone, 5-ethyl-dihydro, 4-Ethyl benzaldehyde, butanoic acid, 2-met, 1-isopropyl-2-methoxy -4-methylbenzene, linalool oxide III, benzenamine, 4-ethoxy-, linalool oxide IV, methyl salicylate, nerol, benzoic acid, 4-ethyl, methyl ester 3,4-dimethyl, acetophenone, benzoic acid, 4-formyl-, methylester, O-diacetylbenzene, geraniol, 4-ethyl acetophenone, benzyl alcohol, 2-phenylethanol, methyl cinamate, hexanoic acid, benzeneacetonitrile, jasmone, 3-hexenyl ester, 3,7-octadien-2, 6-diol, 2,6-dimethyl-, ethanone, 1-(1H-pyrrol-2-yl)-, 4-(1-hydroxyethyl) benzaldehyde, 4-hydroxy-3-methylacetophenone, phenol, 1H-pyrrole-2-carboxaldehyde, furaneol, 1,6-octadiene-3, 5-diol, 3,7-dimethyl-, 4-hexenoic acid, Benzenemethamine, 2-Phenylethyl benzoate, 2H-Pyran-2-one, tetrahydro-6-ethyl, undecanoic acid, methyl ester, jasmone lactone, formate, (Z)-, methylethylmaleimide, 1H-benzotriazole, 1-ethenyl-, dihydroactinidiolide, 1,7-octadien-3, 6-diol, 2,6-dimethyl-, methyl jasmonate, coumaran, coumarin, indole, phenol, 4-propyl-, benzaldehyde and 4-hydroxy. Forty two volatiles were found in green tea, 70 volatiles in oolong tea and 57 volatiles in black tea. Moreover, the volatiles, aliphatics, aromatics and terpenoids increased with increasing degree of fermentation. Jasmine lactone and indole were highest volatiles in oolong teas.

Sheibani *et al.* (2015) studied the changes in flavor volatile composition of oolong tea during tea processing after panning. In this study they selected un-panned Jin Xuan (Chin-Hsuan, or Zhu Shan) oolong tea, which is a cultivar selected in Taiwan in the 1980s for its aromatic flavor. Samples from three batches, purchased from Tea of Life® Health Inc. in Rosedale, New York, were stored at room temperature. Six hundred and eighty grams of oolong tea was placed on a metal baking dish before heated or panned in a convection oven at 120°C for 6 h. The method and selected condition for the panning process were based on our preliminary study of literature. After heating, the tea leaves were left to cool at room temperature. The sample was divided into three batches, which were then panned and analyzed for flavor for each batch separately. Volatile compounds were extracted by SPME for GC-MS analysis. 4 g of samples were brewed in 200 mL of distilled water at 98°C for 5 min then 5 mL of the filtered tea infusion and 1 g of NaCl were placed into 10 mL headspace vials with Teflon-lined silicon septa. SPME was used to extract volatiles and volatiles were analyzed by injection into the GC-MS. Samples were equilibrated for 2

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min prior to extraction. A DVM/Carboxen/ PDMS SPME fiber (2 cm 50/30 μm) was exposed to the headspace above the tea extract in glass vials for 30 min at 40°C with an agitation speed of 250 rpm. Analysis was by using a Shimadzu GCMS-QP2010 using SPME, which showed 48 volatile compounds, including 8 alcohols, 8 aldehydes, 4 ketones, 7 acids, and 11 terpenes. The volatile compounds with the highest peak area were indole (9.3%), furfural (7.1%), 1-ethyl-1-pyrrole-2-carboxaldehyde (5.5%), benzyl nitrile (4.1%), 1,1,5-trimethyl-1,2-dihydronaphthalene (TDN) (3.4%), 4-methoxy-2-methylbenzenamine (2.9%), 5,9,9-trimethyl-spiro[3.6]deca-5,7-dien-1-one (2.8%), methoxyphenyloxime (2.5%), 3,4-dimethylcyclohexanol (2.4%), and 3-amino-4-methylbenzyl alcohol (2.4%). Indole (44.7% of total alcohols) was the most abundant alcohol for both panned and un-panned teas.

Gong *et al.* (2017) identified aroma active compounds, by GC-MS, in Longjing tea (a variety of pan-roasted green tea developed in China) from Hangzhou, Qiantang and Yuezhou provinces. Longjing tea samples were collected as first grade with one bud and one leaf then kept in aluminum foil packages at 4°C in refrigerator until they were analyzed. For brewing 30 g of Longjing tea was kept for 8 min in 800 mL of distilled water at 80°C. The tea infusion was placed in a 20 mL vial, sealed with Teflon covers and equilibrated at 80°C in a water bath with shaking at regular intervals while being exposed to SPME (Solid Phase Microextraction) fibers. Then the SPME fiber was introduced to the GC injector at 250°C for 3 min. The conditions of SPME fibers were (65 μm PDMS/DVB, 80 μm PA, 100 μm PDMS, 75 μm CAR/PDMS and 50/30 μm CAR-PDMS-DVB fiber) and six extraction times (5, 10, 15, 20, 25 and 30) were evaluated. Five fibers were coated and extracted aroma compounds, the adsorbing capacity depends on the characteristics of the coating material. Hence the 50/30 μm CAR-PDMS-DVB fiber is selected in experiment. The reason for the maximum value might be that the aroma compounds gradually desorbed from fiber with the extension of extraction time. Thus, the sample was extracted for 20 min. The total ion chromatograms (TIC) of aroma compounds of three Longjing tea samples showed 1-53 peaks which mainly consisted of 2-acetylpyrrole (nutty and herbal aroma), geraniol (floral and rose-like aroma), phenylethyl alcohol, linalool (sweet and woody odor) and 4-vinylphenol (smoky odor as other phenolics such as p-cresol, phenol and 2-methoxy-4-vinylphenol).

Polat *et al.* (2018) compared the effects of the grades and different drying temperature on black tea volatiles. They divided fresh tea leaves into three groups of 32 kg each and dried them at 100, 130 or 160°C and then graded each group according to the Tyler standard mesh size grade I (20 mesh), grade II (12 mesh) or grade III (9 mesh) then analyzed them using the Solid Phase Microextraction SPME method fiber (75 µm film thickness Carboxen/PDMS) for volatile compounds extraction. 1 g of black tea leaves was placed in a 15 mL vial and sealed and then incubated in a water bath at 60°C for 5 min. Volatile compounds were desorbed for 15 min by inserting the SPME fiber into a GC injector port at a temperature of 230°C using a Thermo Finnigan GC Ultra gas chromatography mass spectrometer. They investigated volatile compounds adsorbed with SPME and having the 50 largest area detected by GC-MS. Intersecting at all temperatures and grades, 36 volatiles were identified that consist of 18 aldehyde, 3 ketone, 2 furan, 5 terpene, 2 hydrocarbon, 3 alcohol and 1 acid, lactone and ester. Furans (6.26%), hydrocarbons (8.50%), alcohols (3.62%), and esters (0.63%) were at most 100°C; terpenes (7.21%) and acids (1.61%) were at most 130°C; aldehyde (69.02%), ketones (8.85%), and lactones (1.45%) were detected at most 160°C drying temperature. The highest flavor index was obtained at 130°C as 0.214. Aldehydes were the highest share of volatile compounds which decreased the flavor index value. The aroma composition affected the grade; however, temperature was more influential on volatile compounds than on grades.

CHAPTER 3

MATERIALS AND METHODS

3.1 Raw materials

3.1.1 The upland black waxy rice cultivar Damgatondam (DGTD) was organically grown between September to October 2016 in a greenhouse at King Mongkut's Institute of Technology Ladkrabang (KMITL) Prince of Chumphon Campus in Chumphon province in southern Thailand. The DGTD rice grass was harvested, about 1 month after planting by cutting it about 1 cm above soil level. The DGTD rice grass was washed with tap water, sealed in a polyethylene bags and wrapped in newspaper and stored in an insulated box containing ice over night for transport from Chumpon province to Faculty of Agro-Industry laboratory, KMITL. The transportation was about 7 h. The external temperature during transport was about 30°C, but the temperature in the box was maintained at 3 - 5°C. On arrival the rice grass was kept in refrigerator at 3-5°C and started the process immediately. The DGTD rice grass was started and finished the process in 1 day.

3.1.2 Green tea Sakurajima brand (Organic Bancha), Japan, tea leaves

3.1.3 Green tea Thai royal project brand (Thailand), tea leaves

3.1.4 Green tea Fujian (Lung Ching) brand, China, tea leaves

3.1.5 Green tea Ujinotsuyu brand (Sencha) Japan, tea bag

3.1.6 Green tea Dilmah brand (Organic Sencha) Sri Lanka, tea bag

3.1.7 Green tea Twining brand, Poland (pure green tea), tea bag

3.1.8 Green tea, Zen brand, China, tea bag

3.2 Chemicals

3.2.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich Corp., St. Louis, MO, USA)

3.2.2 2,2- azinobis (3-ethylbenzothiazoline-6sulfonic acid) diammonium salt (ABTS) (Sigma Aldrich Corp., St. Louis, MO, USA)

3.2.3 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) -(Sigma Aldrich Corp., St. Louis, MO, USA)

3.2.4 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma Aldrich Corp., St. Louis, MO, USA)

3.2.5 Iron (III) chloride hexahydrate (Sigma Aldrich Corp., St. Louis, MO, USA)

3.2.6 Gallic acid monohydrate (Sigma Aldrich Corp., St. Louis, MO, USA)

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- 3.2.7 Quaiacol (Sigma Aldrich Corp., St. Louis, MO, USA)
- 3.2.8 Folin-Ciocalteu reagent (Sigma Aldrich Corp., St. Louis, MO, USA)
- 3.2.9 Sodium acetate trihydrate (Merck KGaA, Darmstadt, Germany)
- 3.2.10 Sodium carbonate (Merck KGaA, Darmstadt, Germany)
- 3.2.11 Potassium persulfate (Merck KGaA, Darmstadt, Germany)
- 3.2.12 96% ethanol (Merck KGaA, Darmstadt, Germany)
- 3.2.13 95.5% methanol (Merck KGaA, Darmstadt, Germany)
- 3.2.14 Hydrochloric acid (HCl) (RCI Labscan Co., Ltd., Rd., BKK, TH)
- 3.2.15 Acetic acid (RCI Labscan Co., Ltd., Rd., BKK, TH)
- 3.2.16 1,3 Dichlorobenzene 98% (Sigma Aldrich Corp., St. Louis, MO, USA)
- 3.2.17 C7-C30 Saturated Alkanes certified material (Sigma Aldrich Corp., MO, USA)

3.3 Instruments and equipments

- 3.3.1 UV-visible spectrophotometer (UV-1800 Shimadzu, Japan)
- 3.3.2 Gas Chromatography (Agilent 6890n, USA) Mass Spectrometer (Agilent 5973, USA)
- 3.3.3 HP-5MS (60 m length x 0.25 mm i.d. x 0.25 μ m film thickness; J&W Scientific) (Supelco, Bellafonte, PA, USA)
- 3.3.4 65 μ m PDMS/DVB SPME fibers
- 3.3.5 Hunter Lab (ColorQuest XE, USA)
- 3.3.6 Chroma-meter (CR-300 Konica Minolta, Japan)
- 3.3.7 Freezer (Sanden Intercool, Thailand)
- 3.3.8 Tray dryer (Progress electronic Ltd., part Thailand)
- 3.3.9 Centrifuge (Hurmle Z206A, Germany)
- 3.3.10 Water activity meter (Aqua Lab 4TE, USA)
- 3.3.11 Hot air dryer (Binder, Germany)
- 3.3.12 Tea pot (Toshiba PLK-30, Japan)
- 3.3.13 Stainless steel steamer (Crocodile cooker, Thailand)
- 3.3.14 Micropipette 0.1, 1 and 5 mL (Rainin, USA)
- 3.3.15 Water bath (Mettler, Germany)
- 3.3.16 Vortex mixer (Genie 2, USA)
- 3.4.17 Test tube (Pyrex, USA)
- 3.4.18 Aluminum foil (3M, Thailand)

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3.4.19 Homogenizer (WiseTis HG-15A, Germany)

3.4 Methods

3.4.1 Sample preparation

Sample from 3.1.1 was washed again and cut into 1 cm long piece for the process.

3.4.2 Process optimization of DGTD tea

3.4.2.1 Steaming process

The steaming process was applied to inhibit enzyme peroxidase of sample. The amount of DGTD rice grass leaves and steaming time were studied.

a. Determination the optimum amount of DGTD rice grass leaves to steam

The entire process used for making DGTD tea was shown in Figure 3.1(Appendix A). Enzyme inhibition was the most crucial step in the process in order to retard the loss of green color in the rice grass leaves. Varied amount of DGTD rice grass leaves 100 g or 0.005 g/cm³, 200 g or 0.010 g/cm³, 300 g or 0.015 g/cm³ or 400 g or 0.020 g/cm³ was studied and put in a single stainless steamer (45 cm diameter) with a steaming time of 60, 90 or 120 sec. The rice grass was spread evenly over the steamer racks so that the thickness of the layer was approximately equal. Clean cheesecloth was placed on the bottom of the rack before spreading the DGTD rice grass leaves. The effects of using a single or double steamer rack with DGTD rice grass leaves on each rack were tested. The accepted DGTD rice grass leaves should be dark green without damage and was judged by visual examination.

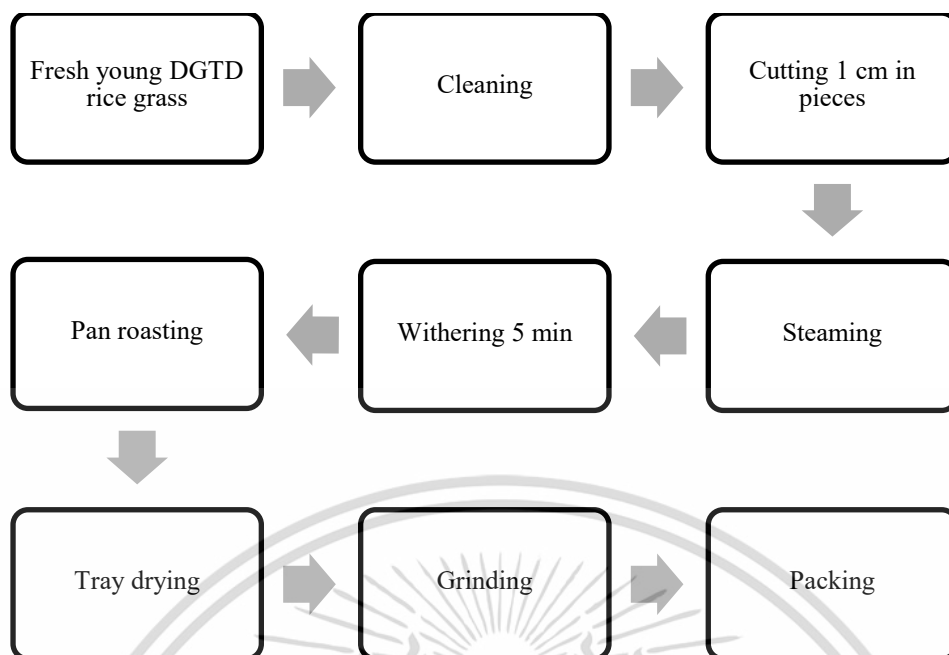


Figure 3.1 Steps of DGTD tea process

b. Test for optimum steaming time to inhibit peroxidase enzyme activity

The optimum amount of DGTD rice grass leaves samples achieved from 3.4.2.1 *a.* was steamed for 30, 45, 60, 75, 90, or 105 sec, and then the peroxidase activity was measured following the method of Alvarez *et al.* (2015). Samples were chopped and homogenized with 30 mL of distilled water in a homogenizer at high speed for 3 min at 4°C. The slurry was filtered through two layers of cheesecloth and centrifuged for 15 min at 10,000 rpm at 4°C. This extract was used for the enzyme source experiment. Peroxidase activity was determined with a UV-Visible spectrometer at 470 nm at 25°C. In brief, the substrate mixture contained 10 mL of 0.01 mL/mL quaiacol solution, 10 mL of 3 mg/mL hydrogen peroxide solution and 100 mL of 0.05 mol/L sodium phosphate buffer (pH 6.5). The reaction cuvette contained 2.9 mL substrate mixture and 0.1 mL crude extract in a total volume of 3 mL. The blank sample (control) contained only 3 mL of substrate mixture. The determination was measured triplicate.

3.4.2.2 Effect of dehydration step on moisture content and bioactive compounds of rice grass leaves

a. Determination the optimum pan roasting condition (First step of dehydration)

After steaming samples with the condition achieved from 3.4.2.1 *b.*, the samples were withered for 5 min by evenly spreading on an aluminum tray at room temperature (about 25°C) and then pan roasting at 70°C for 5, 10, 15, 20, 25 or 30 min. Moisture content was monitored following AOAC (2016). The phenolic content, was measured by the method described by Yun *et al.* (2015) and the antioxidant activity by FRAP, DPPH and ABTS assays, were also determined following the method as described by Wu *et al.* (2003) and explained in section 3.5.3-3.5.6. The experiments were conducted in triplicate.

b. Determination the optimum tray drying condition (Second step of dehydration)

After pan roasting samples with the condition achieved from 3.4.2.2 *a.*, the DGTG rice grass leaves were dried in an electrical cabinet tray dryer at 60°C for 30, 45, 60 or 90 min. Moisture content (AOAC 2016), total phenolic content, and antioxidant activity by FRAP, DPPH and ABTS assays, were also determined following in section 3.5.3-3.5.6.

The completely randomized design (CRD) in a 6 × 4 factorial was applied. All analysis samples were performed in triplicate. Duncan's multiple range tests was used to compare the differences between means and ANOVA used to see the significant effect; $p < 0.05$ was considered as significant.

3.4.3 Proximate analysis, TPC and antioxidant activities of the DGTD tea

The sample of DGTD tea product with optimum condition in this experiment from 3.4.2 that presented the best results were analyzed for TPC described by Yun *et al.* (2015), antioxidant activities (FRAP, ABTS and ABTS assays) described by Wu *et al.* (2003) were also determined following in section 3.5.3-3.5.6, water activity, moisture content, ash, protein, fat, total carbohydrate and fiber and several key minerals; Ca, Na, Mg, K, Fe, and Mn using sub components 978.18, 925.10, 945.38, 981.10, 922.06, 978.10, 984.27, 984.27, 984.27, 999.10 and 984.27, respectively (AOAC, 2016).

3.4.4 Sensory descriptive analysis of DGTD tea

3.4.4.1 Panel selection

Ten trained panelists, aged between 25 and 32 years, were selected from 20 food science postgraduate students in Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang. Trained panels were selected by using a taste and aroma matching and description test. The description taste test was conducted on the candidates' sensory impression of the test object in terms of their appearance (clear and color), flavor and aroma. As proposed by Meilgaard *et al.* (1999) only candidates that were able to describe 80% of the stimuli and describe the characteristics of the stimuli were included.

3.4.4.2 Descriptive analysis test

In order for the panelists (N=10) to become familiar with the attributes of green tea, training samples from 7 brands of commercial green tea (*Camillia sinensis*) from China, Japan, Poland, Sri Lanka and Thailand, which are available in the Thai market both as tea bags and tea leaves, were used. Each of the panelists received a spit cup for expectoration, paper napkins, drinking water and tea samples. Then they were asked to describe the attributes which included appearance, odor, flavor and taste of the commercial products, give definition for each attribute and then also identify reference standards by consensus (Stone and Sidel, 2004). The panelists scored attributes on a 0 to 15 numerical scale with one significant digit for a reference standard that was decided through a general agreement between the panelists. The panelists discussed the attributes and agreed on common description for the samples and then rated the attributed scores with standard deviation (SD) of the mean score of less than 1 after training. After completing the training program, the same group of panelists received 50 mL of the prepared DGTD tea sample in a white cup and evaluated the sample and described its attributes. The samples used for the panel tests were prepared by brewing 6 g of DGTD tea with 450 mL of (98°C) and kept in vacuum flask for just a few minutes, then served to panelists when the temperature was about 60°C.

The completely randomized design (CRD) was applied. All analysis samples were performed in triplicate. Duncan's multiple range tests was used to compare the different of means and ANOVA used to see the significant effect; $p < 0.05$ was considered as significant.

3.4.5 Effect of brewing parameters (tea weight and temperature) on color, bioactive compounds and sensory acceptability

Samples of DGTD tea of (1, 1.5 and 2g) were packed into tea bags and kept in laminated aluminum bags at room temperature until brewing. Tea bags were steeped in 150 mL of 80, 90 or 98°C water for 3 min, thereafter tea bags were removed and the tea liquor was kept in a vacuum flask until the temperature was around 60°C. The sample was measured the properties as described below.

3.4.5.1 Color of brewed tea

Color of DGTD tea was measured by Hunter Lab (ColorQuest XE) colorimeter and reported in L^* (lightness), a^* (redness), b^* (yellowness), hue and chroma values.

3.4.5.2 Bioactive compounds

The brewed DGTD tea beverage samples were analyzed for total phenolic content and antioxidant activity (FRAP, DPPH and ABTS assays) following the method as described by Yun *et al.* (2015) and Wu *et al.* (2003) mentioned in 3.5.3-3.5.6.

3.4.5.3 Sensory acceptability

The brewed DGTD tea beverage samples were served at temperature around 60°C. Fifty panels which were selected from university faculties, government departments and private companies who normally drink hot green tea. The testing parameters were color, clearness, odor, flavor, taste and overall liking by using 9 point hedonic scale (9- like extremely, and 1- dislike extremely).

The completely randomized design (CRD) was applied in section 3.4.5.1 and 3.4.5.2. The randomized completely block design (RCBD) was applied in section 3.4.5.3. All analysis samples were performed in triplicate. Duncan's multiple range tests was used to compare the different of means and ANOVA used to see the significant effect; $p < 0.05$ was considered as significant.

3.4.6 Identification of Volatile compound in DGTD tea by gas chromatography using solid phase microextraction

3.4.6.1 Sample preparation and solid phase microextraction absorption of aroma compound

2 g of DGTD tea were placed in a tea pot and 150 mL of 98°C distilled water was added. The tea was brewed for 3 min. Then the tea was poured through a porcelain strainer into a pre-warmed porcelain bowl. In order to minimize the loss of aroma compound, 0.1g of 1,3-dichlorobenzene (100mg/kg) was added to the tea infusion (5g), as an internal standard, and 3g of sodium chloride was placed in a 20 mL vial, which was sealed with a Teflon cover and equilibrated at 80°C in a water bath with shaking at regular intervals while being exposed to solid phase micro-extraction (SPME) fiber in the sample headspace. After reaching equilibrium, the SPME fiber was withdrawn and the sample was directly injected into GC injector at 250°C for 3 min. The extraction of volatile compounds was carried out by the HS-SPME method using a PDMS/ DVB fiber, with 65 µm film thickness. Before analysis the fiber was preconditioned for 5 min in the injection port of the GC as indicated by the manufacturer.

3.4.6.2 Gas chromatography (GC-MS) analysis of aroma compound

Gas chromatography (GC) conditions were used the method of Lv *et al.* (2012). The gas chromatograph (Agilent-6890n) directly interfaced with Agilent HP 5973 ion trap mass spectrometer (Agilent, USA). The injector temperature, was 250°C and the injection mode was splitless using HP-5MS (60 m length × 0.25 mm i.d. × 0.25 µm film thickness). The oven temperature was programmed as follows: 50°C held for 5 min, then increased to 125°C at a rate of 3°C/min, then held for 3 min and then to 230°C at a rate of 15°C/min. The carrier gas was helium (purity > 99.999%) at a constant flow velocity of 1 mL/min. Mass spectrometer conditions were: ionization mode, EI electron energy 70 eV; interface temperature 280°C, ion source temperature 230°C, quadrupole temperature, 150°C, mass scan range 35-400 AMU and emission current, 34.6 µA. Each compound was identified using the National Institute of Standard and Technology (NIST) library (98 L). The linear retention indices (LRI) were determined via sample injection with a homologous series of alkanes mixture (C7-C30) (Sigma-Aldrich, USA).

3.4.6.3 Data processing

Chromatogram peak, each compound was identified using the National Institute of Standard a Technology (NIST) library (98L) and linear retention indices (LRI) were calculated and compare to previous literature. The relative content of the volatiles compound was obtained using the following equation:

$$\text{Relative content (\%)} = (\text{single constituent area} / \text{total area}) \times 100$$

The Linear Retention Index (LRI) of each compound was obtained using 1 μL n-alkane mixture (C7-C30) (Sigma-Aldrich, USA) under the same experiment condition. The LRI of the volatiles compound was obtained using the following method:

$$\text{LRI} = [100(\text{B}-\text{A})/(\text{C}-\text{A})] + 100 \text{ (carbon of A)}$$

A= retention time of alkane (n-1)

B= retention time of unknown

C= retention time of alkane (n)

3.4.7 Effect of storage time on physical property, bioactive compound, biological quality and sensory acceptance of DGTD tea

The DGTD tea stored in laminated aluminum bags for 12 months was randomly sample after 0, 4, 8, 10 and 12 months of storage to determine properties below:

3.4.7.1 Physical properties, moisture content, water activity and color of DGTD tea were determined. The kept samples were analyzed for moisture content using the hot air oven method at 105°C for 3h (AOAC, 2016). Water activity (A_w) was measured using water activity meter. For color the CIE system was used to measure L^* , a^* and b^* by using a Chroma-meter.

3.4.7.2 Bioactive compounds

The total phenolic content and antioxidant activity (FRAP, DPPH and ABTS assays) were determined for DGTD tea samples with the same method mentioned in 3.5.3-3.5.6.

3.4.7.3 Aerobic plate count

The aerobic plate count was carried out according to FDA-BAM (2001). 25 g of the stored tea sample were placed in sterile plastic bag, diluted with 225 mL of Butterfield's phosphate buffer then blended in a blender. 9 mL of Butterfield's phosphate buffer was

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diluted ten-fold then a micropipette was used to suck 1 mL of each sample at different rates and put on the plate. 15 mL of plate count agar was added (while at 43-45°C). When the plate count agar was formed the plate was turned over then put into an incubator at 37°C for 48 h. The colonies on the plate were counted (30-300 colonies) and calculated in cfu/g.

3.4.7.4 Yeast and mold

Assessment of yeast and mold counts were carried out according to FDA-BAM (2001). 25 g of stored tea sample were placed in a sterilized plastic bag, diluted with 225 mL of Butterfield's phosphate buffer then blended in a blender. 9 mL of the Butterfield's phosphate buffered solution was used to prepare a ten-fold dilution then 1 mL each sample was micropipetted at different rate and put on a plate. 1 mL of 10% tartaric acid was added (while at 43-45°C) into 15 mL of potato dextrose agar. When the potato dextrose agar was formed the plate was turned over then put into an incubator at 37°C for 48 h. The colonies were counted on the potato dextrose agar and calculated in cfu/g.

3.4.7.5 Sensory evaluation

Stored samples were brewed and evaluated for their sensory acceptability. Thirty people constituted the panel, which were selected from university faculties, government departments and companies who normally drink hot green tea. Brewed teas were served and the testing parameters were color, clearness, odor, flavor, taste and overall liking by using 9-point hedonic scale (9- like extremely, and 1-dislike extremely).

The completely randomized designs (CRD) were applied in sections 3.4.7.1, 3.4.7.2, 3.4.7.3 and 3.4.7.4. The randomized completely block design (RCBD) were applied in section 3.4.7.5. All analysis samples were performed in triplicate. Duncan's multiple range tests was used to compare the different of means and ANOVA used to see the significant effect; $p < 0.05$ was considered as significant.

3.5 Antioxidant activity analysis

3.5.1 Preparation of DGTD rice grass leaves for analysis

DGTD rice grass leaves samples (2 g) was extracted with 50 mL of methanol that were then thoroughly mixed for 1 min, centrifuged at 4500 rpm for 10 min and kept at -20°C until used in the analyses. All about determination of bioactive compounds of dehydration steps were determined by the method of Yun *et al.* (2015) mention in 3.5.3 for total

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phenolic content and by the methods of Wu *et al.* (2003) mentioned in 3.5.4-3.5.6 for antioxidant activity (FRAP, DPPH and ABTS assays).

3.5.2 Preparation of DGTD tea beverage for analysis

About 1, 1.5 and 2g of samples in tea bags were brewed in 150 mL of 80, 90 or 98°C water for 3 min. 15mL of each tea was stored in darkness in a freezer at -20°C. The DGTD tea beverage samples were used to determine total phenolic content by the method of Yun *et al.* (2015) from 3.5.3 and for antioxidant activity (FRAP, DPPH and ABTS assays) by the method of Wu *et al.* (2003) mentioned in 3.5.4-3.5.6.

3.5.3 Total phenolic content determination

Total phenolic content was determined using the method of Yun *et al.* (2015) where 0.5 mL of extracted sample was added to 5 mL of Folin Ciocalteu's phenol reagent, diluted with distilled-water (1:10) and vortexed. After 5 min, 4 mL of sodium carbonate (1 M) was added and mixed well and incubated at room temperature (25°C) in the dark for 15 min and its absorption was measured at 765 nm. The standard graph from gallic acid solution was used for comparison and the results were reported in mg GA equivalent/g sample (Appendix C1).

3.5.4 Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out according to Wu *et al.* (2003). The FRAP solution was prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM Iron (III) chloride hexahydrate (1:1:1) then incubation at 37°C for 30 min. 0.15 mL of the diluted sample was dropped into a glass tube then 2.85 mL of FRAP solution was added, vortexed and stored for 30 min in room temperature in the dark. Light absorbent at 593 nm was measured and a standard graph from a solution of 1 mM Trolox was plotted and reported in mM Trolox equivalent/g of sample (Appendix C2).

3.5.5 DPPH radical scavenging activity

The DPPH assay was carried out according to Wu *et al.* (2003). 1.5 mL of extracted sample was dropped into a glass tube and 1.5 mL of 0.15 mM DPPH solution in 96% ethanol was added and incubated for 30 min at room temperature in the dark. Light absorption was measured at 517 nm then a standard curve graph was plotted from 1mM Trolox (Appendix C3). The blank was set with distilled water and 99.5% methanol 1:1, then reported as mM Trolox equivalent/g of sample (Appendix C3).

3.5.6 ABTS radical cation decolorization assay

The ABTS assay was carried out according to Wu *et al.* (2003). ABTS reagent was prepared by combining 7.4 mM ABTS solution with 2.6 mM of potassium persulfate solution 1:1. Before testing, it was mixed with 1 mL ABTS reagent and 50 mL of 99.5% methanol then adjusted to the value of light absorption to be 1.1 ± 0.02 by using methanol. 0.15 mL extract sample was dropped into a glass tube and 2.85 mL ABTS reagent was added then incubated for 2 h at room temperature in the dark. Light absorption was measured at 734 nm then plotted against a standard curve from a solution of 1 mM Trolox and reported as mM TE/g sample (Appendix C4).



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CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Process optimization of DGTD tea

4.1.1 Steaming process

a. Optimum amount of DGTD rice grass leaves to steam

Inhibition enzyme activity is the most crucial step in the process in order to retard the loss of green color in the rice leaves. Attempts to use a double rack steamer was not successful because when the double rack steamer was used, there was a possibility of insufficient heat for enzyme inactivation in the upper rack while damage could occur simultaneously to the sample on the lower rack. Using a single rack steamer resulted in more consistent quality of the rice leaves due to a better temperature distribution. Among the sample sizes and steaming times tested, the optimum was the combination of 200 g for 60 sec (Table 4.1) using a single rack steamer, 60sec was the minimum steaming time that inhibited peroxidase activity (Figure 4.1). Steamed samples of 300 g and 400 g resulted in irregularly color even when steaming for only 60 sec and samples of 100 g gave similar results to 200 g. All sample sizes resulted in some damages when steamed for 90 or 120 sec.

Table 4.1 Characteristics of DGTd rice grass leaves with different weights and steaming times

Tea weight (g)	Steaming time (sec)	Characteristics
100	60	Dark green, without damage
100	90	Damaged
100	120	Damaged
200	60	Dark green, without damage
200	90	Dark green, with damage
200	120	Damaged
300	60	Irregularly color
300	90	Dark green, with damage
300	120	Dark green, with damage
400	60	Irregularly color
400	90	Dark green, with damage
400	120	Dark green, with damage

b. Optimum steaming time to inhibit peroxidase enzyme activity

A sharp decrease in peroxidase activity levels with increasing steaming times over the range of 30 and 45 sec was observed. Peroxidase activity was completely inhibited after steaming for 60 sec (Figure 4.1). Precise steaming time is essential in producing an acceptable product since browning can occur, as observed in the experiments, due to the tissue and the chlorophyll being damaged by over exposure to heat or by enzyme action on the polyphenols because the temperature was insufficient to deactivate them. Therefore, on balance, it was concluded that the most suitable steaming time for DGTd tea was 60 sec at $98 \pm 2^\circ\text{C}$, which is consistent with the results described by Wang *et al.* (2000) for *C. sinensis* tea.

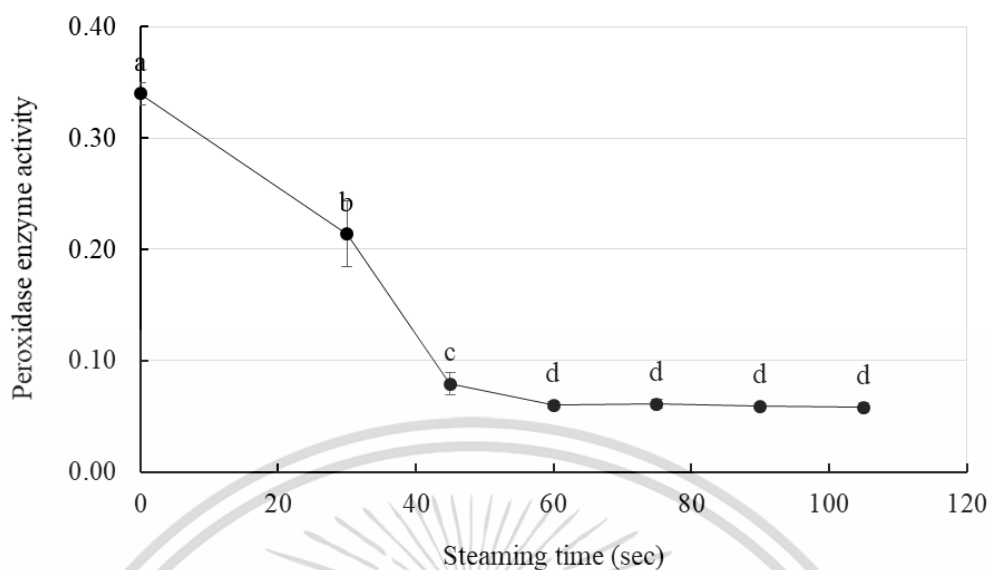


Figure 4.1 Effect of steaming time on peroxidase enzyme activity

Note: ^{a,b,c,d}, the different small letter in line was significantly different at $p < 0.05$.

4.1.2 Effect of dehydration step on moisture content and bioactive compounds of DGTD rice grass leaves

a. Determination the optimum pan roasting condition (First step of dehydration)

After 5 min of withering, the moisture content of DGTD rice grass leaves was approximately 70%, which then continuously and sharply decreased during pan roasting for 5, 10 or 15 min giving moisture contents of 54.0, 44.3 and 37.1%, respectively. Subsequently, the moisture content continued to decrease then leveled off (Figure 4.2). During pan roasting for up to 25 min the leaves still had a fresh green color but this was not consistent after drying. After roasting for 35 min the moisture content was about 36%, which was not significantly different ($p \geq 0.05$) from 30 min but gave the leaves a green, dry, crisp appearance but some parts of leaves were burnt. It was considered that pan roasting at $70 \pm 2^\circ\text{C}$ for 30 min was the most effective since it gave the leaves a curled appearance and a mild roasted smell of tea. As Uhl (2016) described, pan frying is traditionally done by hand on a wok over an open flame. The contact between the leaves and the hot metal triggers the Maillard reaction to form molecules that create unique odors and flavors. The Maillard reaction is a reaction between reducing sugars and amino acids at high temperature, which produces brown pigments that can have a caramel flavor.

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Kawakami and Yamanishi, (1999), Choi (2010) and Zheng *et al.* (2016) reported that green teas, which are prepared by roasting or pan frying, contain high levels of millard reaction products, such as 1-ethyl-3, 4-dehydropyrrolidone, pyrazines, pyrroles, pyrans and furans. Pyrazines and 1-ethyl-3, 4-dehydropyrrolidone play an important role in developing a roasted flavor in both roasted and pan-fried green teas.

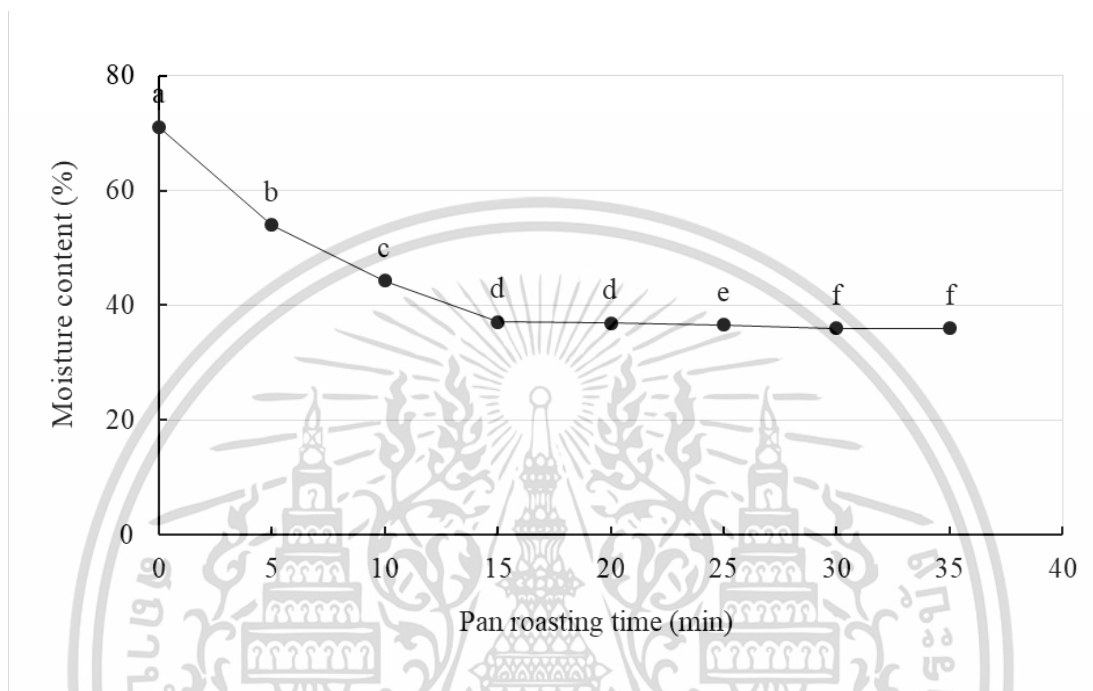


Figure 4.2 Moisture content of DGTD rice grass leaves during pan roasting at 70°C

Note: ^{a,b,c,d,e,f}, the different small letter in line was significantly different at $p < 0.05$.

Total phenolic content and antioxidant activity of pan roasted samples progressively and continuously decreased significantly ($p < 0.05$) with increased heating time. The TPC decreased from 15.42 to 14.29 mg GAE/g sample, FRAP decreased from 533.67 to 516.57 mM TE/g sample, DPPH decreased from 53.96 to 47.81 mM TE/g sample and ABTS decreased from 178 to 150.22 mM TE/g sample (Table 4.2). Lorsuwan *et al.* (2008) reported that there are many factors that directly affect the stability of phenolic compounds particularly temperature and acidity, which caused total phenolic content to decrease. Pan roasting and other thermal processes can destroy antioxidant and phenolic compounds. Zzaman *et al.* (2013) showed that roasting cocoa beans with superheated steam at temperatures of 150, 200 or 250°C and times over the range of 10 - 50 min resulted in significantly ($p < 0.05$) lower free radical scavenging activity, antioxidant properties, total phenols and total flavonoids with the higher temperatures and longer times.

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Table 4.2 Total phenolic content (TPC) and antioxidant activities (FRAP, DPPH and ABTS assays) of DGTG rice grass leaves during pan roasting

Pan roasting time (min)	TPC (mg GAE/g sample)	FRAP (mM TE/g sample)	DPPH (mM TE/g sample)	ABTS (mM TE/g sample)
5	15.42 ^a ±0.27	533.67 ^a ±5.17	53.96 ^a ±1.46	178.00 ^a ±4.16
10	15.36 ^a ±0.07	529.14 ^{ab} ±4.68	53.50 ^a ±0.47	161.56 ^b ±7.73
15	15.21 ^a ±0.10	527.24 ^{ab} ±5.07	51.94 ^a ±0.49	160.89 ^b ±2.14
20	14.74 ^b ±0.06	521.76 ^{bc} ±3.67	49.60 ^b ±1.20	155.56 ^{bc} ±1.02
25	14.62 ^b ±0.12	521.53 ^{bc} ±6.07	48.58 ^b ±0.84	151.78 ^c ±1.68
30	14.29 ^c ±0.45	516.57 ^c ±3.60	47.81 ^b ±1.56	150.22 ^c ±3.29

Note: ^{a,b,c} Mean±SD, the different small letter in each column was significantly different at $p < 0.05$.

b. Determination the optimum tray drying condition (Second step of dehydration)

The samples that had been pan roasting for 5, 10 or 15 min and then tray dried at $60 \pm 2^\circ\text{C}$ for 30, 45 or 60 min had moisture contents higher than 5% for all conditions (Figure 4.3), which were higher than the standard for moisture content of dried tea leaves (Temple and Boxtel, 1999; Winyangkul *et al.*, 2007). However, when the drying time was increased to 90 min the moisture content fell below 5%. When samples were tray dried for 60 min combined with pan roasting for 20, 25 or 30 min their moisture contents were 4.97, 4.85 and 4.14% respectively. Using longer drying time had little effect on the moisture content because the equilibrium moisture content of the drying system had been reached. Previously, Keeratiburana and Srijesdaruk (2011) showed the effect of tray drying at 40, 50 or 60°C for 10, 12 or 14 h on moisture content of KDML105 rice grass tea resulted, as would be expected, in decreasing moisture content with increasing temperature and time. However, the dehydration step in DGTG tea processing resulted in stronger development of tea aroma and jasmine rice odor after 90 min of tray drying. Also, the optimum tray drying process achieved a moisture content below 5%, which gave stability and freedom from microorganisms (Winyangkul *et al.*, 2007).

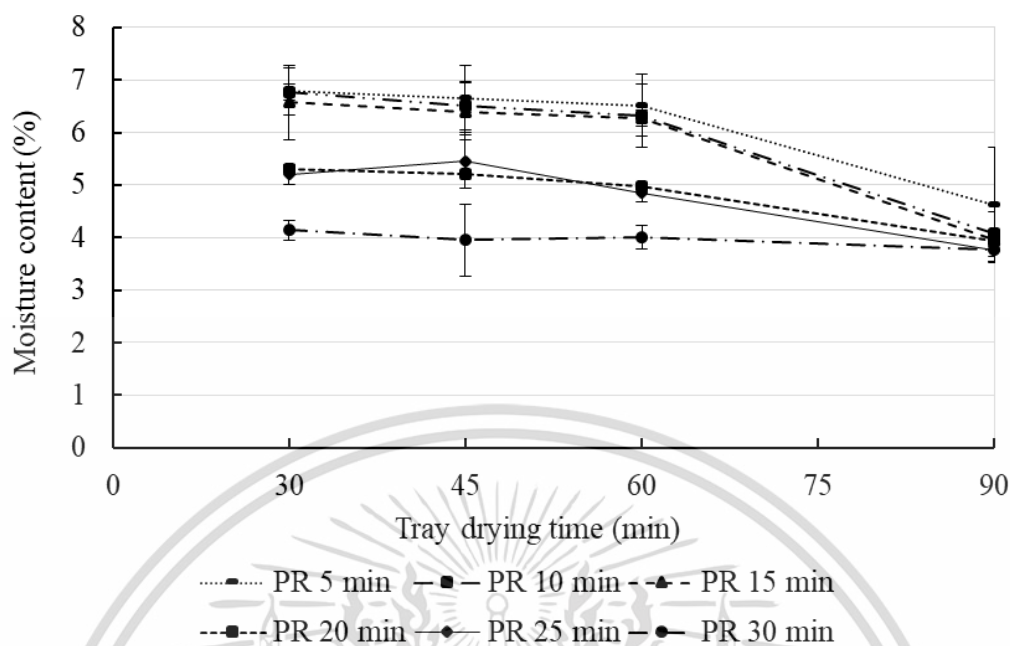


Figure 4.3 Moisture content of DGTD rice leaves leaves pan roasted (PR) for 5, 10, 15, 20, 25 or 30 min prior to dehydration in an electrical cabinet tray dryer (TD) at 60°C for 30, 45, 60 or 90 min.

The effects of pan roasting DGTD rice grass leaves for 30 min and tray drying in an electrical cabinet tray dryer at 60°C for 30, 45, 60 or 90 min on TPC, FRAP, DPPH and ABTS antioxidant activity were investigated. The TPC levels were 13.98, 13.09, 10.46 and 9.08 mg GAE/g, the FRAP levels were 498.34, 449.27, 424.64 and 369.90 mM TE/g, the DPPH levels were 45.65, 41.33, 36.73 and 29.17 mM TE/g and the ABTS levels were 148.06, 144.89, 133.78 and 100.22 mM TE/g, respectively after drying at 60°C for 30, 45, 60 or 90 min (Figure 4.4). The decreasing effect in TPC and FRAP with increasing drying time is consistent with the effects reported on mortino fruit (*Vaccinium floribundum*) by Lopez-Vidana *et al.* (2016) who found that using temperature of 40, 50 or 60°C for 15, 30, 45, 60, 90, 120 180, 240 or 300 min resulted in lower antioxidant capacity and TPC when drying with increasing time. Also, Keeratiburana and Srijesdaruk (2011) showed that tray drying KDML (Khao dawk mali) rice grass at 40, 50 or 60°C for 10, 12 or 14 h resulted in decreased bioactive compounds (DPPH assay) when temperature and time were increased so the lowest temperature and shortest time gave the highest level of bioactive compounds. For chamomile tea, drying at 50°C for 4 or 5 h resulted in both decreased moisture content

and decreased TPC with longer exposure time (Rungsirisakun, 2015). This confirmed that a high temperature for a longer time reduced the determined bioactive compounds.

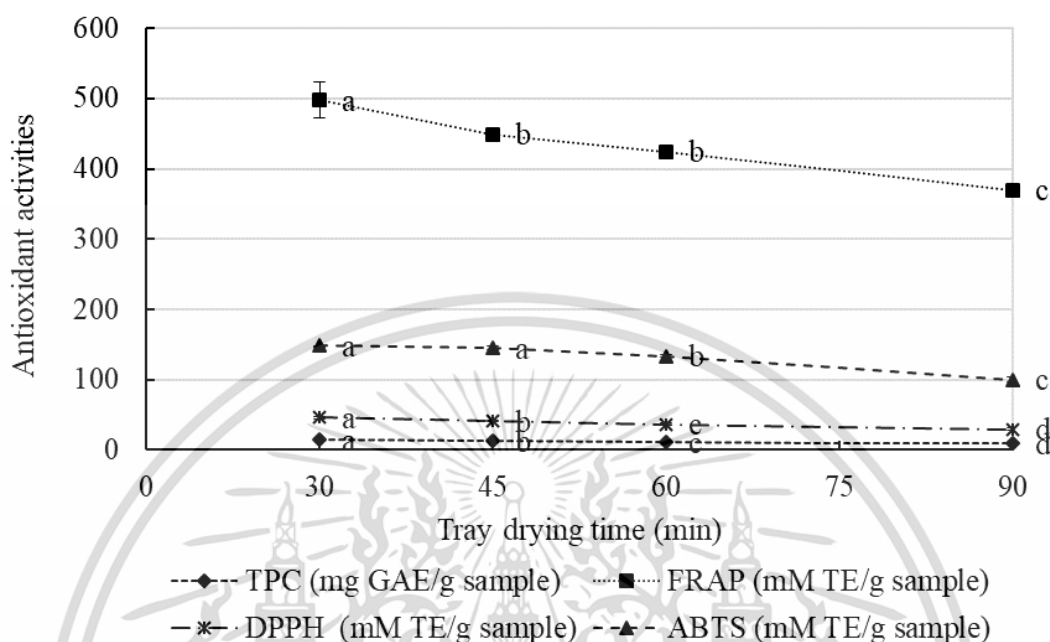


Figure 4.4 Total phenolic content, FRAP, DPPH and ABTS of DGTD rice leaves after pan roasting for 30 min and tray drying at $60 \pm 2^\circ\text{C}$ for 30, 45, 60 or 90 min.

Note: ^{a,b,c,d}, the different small letter in each line was significantly different at $p < 0.05$.

4.2 Proximate analysis, TPC and antioxidant activities of the DGTD tea

The water activity and proximate analysis of DGTD tea, which had been processed by steaming for 60 sec, pan roasting at $70 \pm 2^\circ\text{C}$ for 30 min and tray drying at $60 \pm 2^\circ\text{C}$ for 90 min were compared with several characteristics of tea leaves of *C. sinensis* (Ahmad *et al.*, 2014; Adnan *et al.*, 2013) (Table 4.3). The results were shown to be considerable differences, not only between DGTD tea and *C. sinensis* tea, but also within *C. sinensis*. For example, protein value was 18.06% (Ahmad *et al.*, 2014) and other was 1.23% (Adnan *et al.*, 2013) while DGTD tea was 17.73%. These differences in protein levels may be due to different cultivars and cultivation condition. There were also variations in mineral contents with DGTD tea, which were higher in Mg, K and Na, but lower in Ca, Fe and Mn compared to *C. sinensis* tea (Ahmad *et al.*, 2014; Ramdani *et al.*, 2013) (Table 4.3). However, the mineral content in any crop reflects the soil in which it is grown so the mineral levels in the DGTD tea were comparably acceptable. Although TPC level in the DGTD tea was lower than *C. sinensis* tea (Nor and Fadzelly, 2013; Sheikh *et al.*, 2015)

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(Table 4.3) and antioxidant activity as measured by FRAP, DPPH and ABTS these levels were all considered acceptable (Lee *et al.*, 2014) (Table 4.3).

Table 4.3 Proximate analysis, TPC and antioxidant activities of the DGTD tea and *C. sinensis* tea

Parameters	DGTD	<i>C. sinensis</i> (Ahmad <i>et al.</i> , 2014)	<i>C. sinensis</i> (Adnan <i>et al.</i> , 2013)
Water activity	0.49	-	-
Moisture content (%)	3.77	4.88	6.46
Ash (%)	7.94	5.60	4.57
Crude Fiber (%)	22.59	15.35	16.16
Fat (%)	2.58	2.49	1.68
Protein (%)	17.73	18.06	1.23

Parameters	DGTD	<i>C. sinensis</i> (Ahmad <i>et al.</i> , 2014)	<i>C. sinensis</i> (Ramdani <i>et al.</i> , 2013)
Calcium (Ca) (mg/kg)	3400	3747.5	6699
Iron (Fe) (mg/kg)	66.4	205.3	119
Magnesium (Mg) (mg/kg)	3200	652.2	1993
Manganese (Mn) (mg/kg)	139.1	-	663
Potassium (K) (mg/kg)	20400	17031.2	8095
Sodium (Na) (mg/kg)	113.8	75.5	78.2

Parameters	DGTD	<i>C. sinensis</i> (Nor and Fadzelly, 2013)	<i>C. sinensis</i> (Sheikh <i>et al.</i> , 2015)
Total phenolic content (mg GAE/g)	9.53	72.70	74.51

Parameters	DGTD	<i>C. sinensis</i> (Lee <i>et al.</i> , 2014)	<i>C. sinensis</i>
FRAP (mM TE/g)	357.52	1555.06	-
DPPH (mM TE/g)	34.08	1423.22	-
ABTS (mM TE/g)	108.89	4293.33	-

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4.3 Effect of brewing parameters (tea weight and temperature) on color, bioactive compounds and sensory acceptability of DGTD tea

4.3.1 Color of brewed tea

Effect of tea weight and brewing temperature for preparing DGTD tea on color determination was shown in Table 4.4. It was found that the increment of tea weight and temperature affected L^* decreasing (darker), a^* decreasing (greener), b^* increasing (yellower), chroma increasing (brighter) and hue decreasing (yellower). Kitpot *et al.* (2007) studied the influence of green tea color effected from brewing temperature and time, it was found the similar results that b^* increased, L^* decreased, and a^* decreased when temperature and time were increased. Increasing of DGTD tea weight and temperature affected DGTD tea color because green tea from DGTD rice leaves (Gebely, 2015) which were ground after tray dried, the cells were broken by the blending process resulting in greener of the brewing tea. Generally, there are two kinds of tea leaves pigment; chlorophylls and carotenoids. Carotenoids in tea leaves consist of carotenes (orange color) and xanthophyll (yellow color), which determined color of instant tea leaves. In addition, Othong *et al.* (2014) studied on Yanang (*Tiliacora triandra* (Colebr.) Diels) herbal tea and its physiochemical properties and found that lightness (L^*) was 52.30, redness (a^*) was -0.06 and yellowness (b^*) was 1.36 values depended on many factors including raw material, species, types of tea and processing method.

Table 4.4 Effect of DGTD tea weight and brewing temperature on color values of brewed DGTD tea

Weight of tea (g)	Brewing (°C)	<i>L</i> *	<i>a</i> *	<i>b</i> *	<i>C</i>	<i>H</i>
1	80	97.50 ^{aA} ±0.13	-9.58 ^{aA} ±0.04	31.91 ^{cC} ±0.14	33.60 ^{cC} ±0.16	108.25 ^{aA} ±0.04
	90	96.10 ^{bA} ±0.02	-10.30 ^{bA} ±0.01	32.78 ^{bC} ±0.13	34.15 ^{bC} ±0.13	106.28 ^{bA} ±0.02
	98	95.98 ^{cA} ±0.08	-10.52 ^{cA} ±0.07	35.51 ^{aC} ±0.09	36.98 ^{aC} ±0.09	106.17 ^{cA} ±0.05
1.5	80	95.60 ^{aB} ±0.07	-9.65 ^{aB} ±0.05	38.33 ^{cB} ±0.16	39.81 ^{cB} ±0.16	105.67 ^{aB} ±0.03
	90	95.18 ^{bB} ±0.03	-10.75 ^{bB} ±0.03	41.40 ^{bB} ±0.03	42.86 ^{bB} ±0.04	105.00 ^{bB} ±0.02
	98	93.39 ^{cB} ±0.09	-11.10 ^{cB} ±0.02	43.56 ^{aB} ±0.31	44.61 ^{aB} ±0.31	104.60 ^{cB} ±0.05
2	80	94.99 ^C ±0.18	-11.28 ^{aC} ±0.08	45.38 ^{aA} ±0.54	46.77 ^{aA} ±0.55	103.98 ^{aC} ±0.07
	90	94.47 ^{bC} ±0.08	-11.30 ^{bC} ±0.09	46.08 ^{bA} ±0.08	47.52 ^{bA} ±0.29	103.73 ^{bC} ±0.03
	98	94.19 ^{cC} ±0.10	-12.01 ^{cC} ±0.06	46.17 ^{aA} ±0.28	47.62 ^{aA} ±0.08	102.49 ^{cC} ±0.02

Note: *Values are mean ± standard deviation of three replications.

^{a,b,c} Mean±SD, The different small letter in each temperature was significant different at $p<0.05$.

^{A,B,C} Mean±SD, The different capital letter in each tea weight was significant different at $p<0.05$.

4.3.2 Bioactive compounds

Total phenolic content and antioxidant activities by FRAP, DPPH and ABTS in 1, 1.5 and 2 g of tea brewed at 80, 90 and 98°C were shown in Figure 4.5. The TPC and antioxidant activities by FRAP, DPPH and ABTS assays were significantly ($p < 0.05$) increasing when DGTD tea weight and temperature were increased, the maximum values were for brewing in 2 g tea weight at 98°C (18.27 mg GAE/g, 114.40, 47.14 and 55.44 mM TE/g, respectively). Perva-uzunalic *et al.* (2006) studied different extraction methods of green tea (cultivar Fanning Belas, from China) by different temperature and extraction times. It was confirmed that higher brewing temperature of water also increased total phenolic content. In addition, Komez *et al.* (2010) reported that maximal total phenolic content of tea when brewing at 100°C and antioxidant activities of green tea when brewing at higher temperature were also higher, which related to the initial total phenolic content in tea samples. Liu *et al.* (2017) reported that different temperature of brewing tea showed significantly ($p < 0.05$) different results for antioxidant activity in green tea. It was found that brewing green tea at 100°C caused maximal antioxidant activity and was the best temperature in brewing green tea since it may co-operate with antioxidant activity.

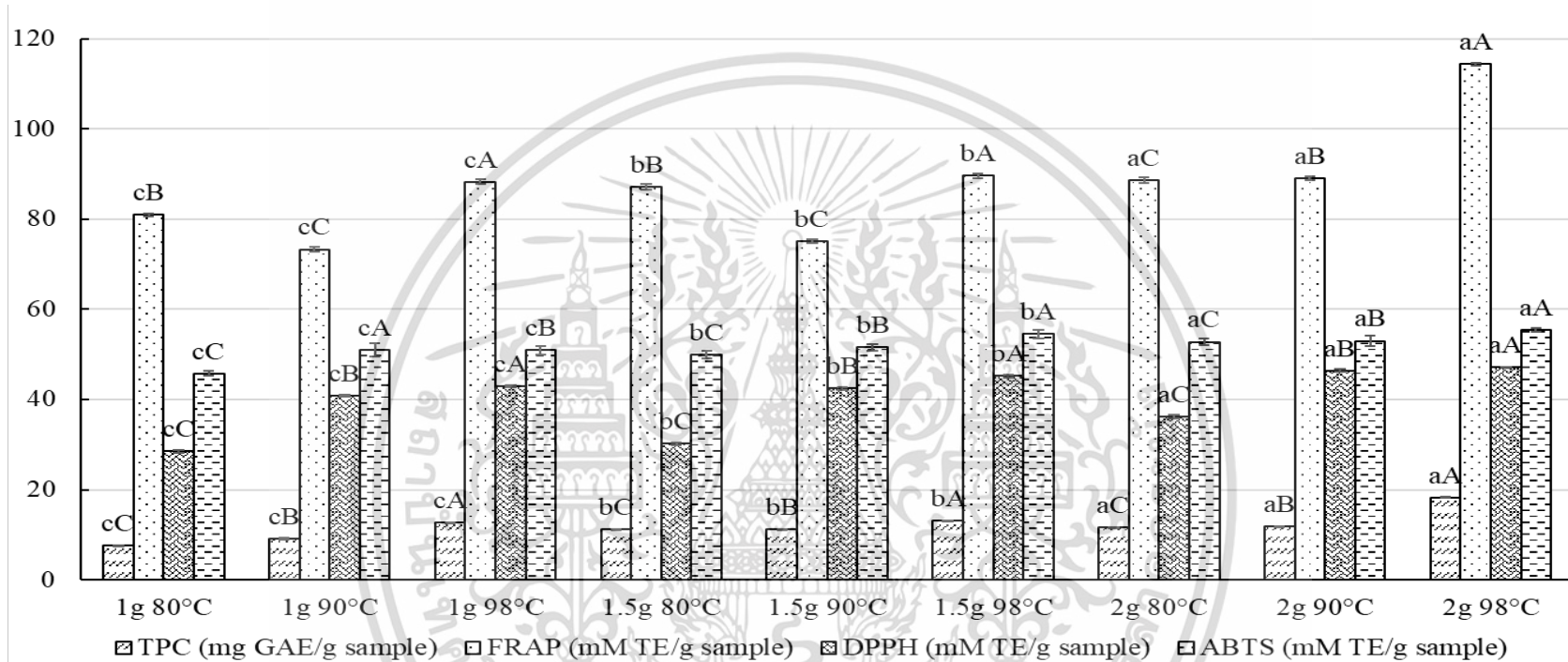


Figure 4.5 Total phenolic content and antioxidant activities of DGTG tea; total phenolic content (mg GAE/g sample). antioxidant activity by FRAP (mM TE/g sample), antioxidant activity by DPPH (mM TE/g sample) and antioxidant activity by ABTS (mM TE/g sample) brewing at 80, 90 and 98°C, with 1, 1.5 and 2 g DGTG tea.

Note: ^{A,B,C}, the different capital letter in each temperature of DGTG tea was significant different at $p < 0.05$.

^{a,b,c}, the different small letter in each weight of DGTG tea was significant different at $p < 0.05$.

4.3.3 Sensory acceptability

Testing the acceptability of DGTD tea by sensory evaluation was necessary because DGTD tea was a novel product, which makes it necessary to determine whether or not the consumer likes and accepts for the product. The proper amount of DGTD tea and brewing method had to be evaluated in order to assess consumer satisfaction. Fifty panelists, who consume hot drink green tea at least 2-3 times per month, were selected to participate in the DGTD tea evaluation. They were served DGTD tea sample brewed from 1, 1.5 or 2g of DGTD tea steeping in 80, 90 or 98°C water. The 9 point hedonic scale test was used to evaluate in color, clearness, odor, flavor, taste and overall liking. Results were shown in Table 4.5. Increasing the weight of DGTD tea and brewing temperature affected the intensity of color both green and yellow, but lightness was decreased (see color determination), however the color of DGTD tea was in the range of green color. For sensory evaluation, the participants were satisfied with increasing the intensity values of the color. For clearness satisfaction, increasing the weight of the DGTD tea samples and temperature resulted in decreasing the clearness (more turbidity), which resulted in increasing of liking. Due to the general characteristics of commercial green tea which participants get used to, since that also showed some turbidity or was not a clearly aqueous. With increasing weight of DGTD tea and temperature, the intensity of the odor also increased. The descriptive analysis of DGTD tea has also been conducted and showed that the particularity sensory odor of rice grass tea from DGTD leaves was jasmine odor, seaweed odor, green odor, and hay odor, which positively affected the liking scores. Flavor is the sensory impression of a food or other substance and is determined by the chemical senses of taste and smell. With increasing weight of DGTD tea and temperature the intensity of flavor also increased. The particularities of flavor of DGTD tea were tea flavor, and seaweed flavor, which affected liking scores when the intensities were increased. With increasing weight of DGTD tea and temperature there was also increase in the intensity of the bitter taste. The participants gave higher taste scores when the characteristic of DGTD tea and tea (*C. Sinensis*) was a bit bitter. The tea produced from *Camellia Sinensis* (commercial tea) always contains catechin that gives it a bitter and astringent taste. As mentioned above, when the weight and temperature were increased during the brewing of DGTD tea, the intensity of each attributes measure and overall liking also increased. The highest acceptability score of the attributes, color, clearness, odor, flavor, taste, and overall liking, from brewing 2 g of DGTD tea at 98°C were 7.24,

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7.67, 7.52, 7.65, 7.82 and 7.79, respectively, and panelists assessments were that they moderately liked the DGTD tea product.



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Table 4.5 Sensory scores of brewed DGTD tea preparing with different weight and brewing temperature.

Weight of tea (g)	Brewing temp. (°C)	Color	Clearness	Odor	Flavor	Taste	Overall liking
1	80	5.48 ^{cc} ±1.09	5.76 ^{cc} ±1.15	5.64 ^{cc} ±1.10	5.69 ^{cc} ±1.02	5.63 ^{cc} ±1.03	5.57 ^{cc} ±1.01
	90	5.82 ^{bc} ±0.99	6.16 ^{bc} ±1.02	6.00 ^{ac} ±1.08	6.12 ^{ac} ±1.02	6.12 ^{ac} ±1.06	6.05 ^{ac} ±1.10
	98	6.02 ^{ac} ±0.89	6.29 ^{ac} ±0.98	5.69 ^{bc} ±0.93	5.82 ^{bc} ±0.97	5.68 ^{bc} ±0.89	5.69 ^{bc} ±0.98
1.5	80	5.96 ^{cb} ±0.94	6.26 ^{cb} ±1.01	6.05 ^{cb} ±0.93	6.24 ^{cb} ±0.98	6.29 ^{cb} ±0.95	6.33 ^{cb} ±1.00
	90	6.25 ^{bb} ±0.94	6.54 ^{bb} ±1.03	6.38 ^{bb} ±0.95	6.52 ^{bb} ±0.88	6.48 ^{bb} ±1.00	6.54 ^{bb} ±0.95
	98	6.60 ^{ab} ±0.85	6.81 ^{ab} ±1.05	6.58 ^{ab} ±1.03	6.76 ^{ab} ±0.99	6.82 ^{ab} ±1.03	6.80 ^{ab} ±1.03
2	80	6.20 ^{ca} ±0.98	6.70 ^{ca} ±0.96	6.52 ^{ca} ±0.89	6.61 ^{ca} ±0.85	6.61 ^{ca} ±0.94	6.59 ^{ca} ±0.90
	90	6.50 ^{ba} ±1.03	6.88 ^{ba} ±0.98	6.78 ^{ba} ±1.04	6.86 ^{ba} ±1.01	6.97 ^{ba} ±1.10	7.01 ^{ba} ±1.03
	98	7.24 ^{aa} ±0.98	7.67 ^{aa} ±0.88	7.52 ^{aa} ±1.02	7.65 ^{aa} ±0.96	7.82 ^{aa} ±0.97	7.79 ^{aa} ±0.89

Note: Values are mean ± standard deviation of three replications.

^{a, b, c} Mean±SD, the different small letter in each temperature of DGTD tea was significant different at p<0.05.

^{A, B, C} Mean±SD the different capital letter in each weight of DGTD tea was significant different at p<0.05.

4.4 Sensory descriptive analysis of DGTD tea

Ten trained panelists described the appearance, attributes and specified definition of all the DGTD tea products, which resulted in 11 different attributes quoted. These attributes were yellowness, clearness, tea odor, hay odor, green odor, seaweed odor, jasmine rice odor, tea flavor, seaweed flavor, bitter taste and astringent taste (Table 4.6). All the attributes were also present in the 7 brands of commercial *C. sinensis* tea that were tested except for jasmine odor which was found only in the DGTD tea. However, Lee and Chambers (2010) described 18 attributes of *C. sinensis* tea produced from China, Japan and Korea as: green, asparagus, celery, green beans, green herb-like, parsley, spinach, brown, ashy, sooty, burnt, scorched, tobacco, animalic, musty, new leather, seaweed, straw like, bitter, astringent and tooth etch. Additionally, Vittayaporn and Chompreeda (2010) studied preference mapping of Thai consumers for commercial green tea with added roasted brown rice and also described the following 18 sensory attributes: yellow color, clear, tea aroma, dry aroma, green, seaweed, roast flavor, bitter, astringent, tea aftertaste, roast after taste, bitter after-taste and astringent after taste.

The intensity and comparison between reference standards, DGTD tea and *C. sinensis* tea (Table 4.7) for yellowness and clearness were very similar, but odor and tea flavor were different. This could be accounted for because oolong tea, which was made from *C. sinensis*, was used as the reference standard and therefore had a more pronounced tea odor and flavor as would be expected. In addition, the intensity of hay odor which was absent in *C. sinensis* but found as a mild odor in DGTD tea. Fresh grass was used as a reference standard for green odor, since DGTD tea is made from rice grass leaves, which are members of the grass family (Ricepedia, 2017). DGTD tea was found to have a stronger green odor than the fresh grass. DGTD tea was quite closed to *C. sinensis* green tea for seaweed odor and seaweed flavor. Japanese green teas often exhibit a fresh and marine aroma, very similar to seaweed since dimethyl sulfide is a compound, found in Japanese green tea that has been described as having a smell of seaweed as well as cooked cabbage, asparagus, sweet corn, and seaweed (Caicedo, 2017). Jasmine rice odor was found in the DGTD tea that gave it a pleasant smell but it was absent in *C. sinensis* tea. When DGTD tea and *C. sinensis* tea was compared for tea flavor, *C. sinensis* tea had a higher score than DGTD tea. For bitter taste and astringent taste, DGTD tea scored less than *C. sinensis* tea, which was consistent with levels of TPC of DGTD tea and *C. sinensis* tea which was

found to have different phenolic compounds. The major polyphenols components in *C.*
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sinensis tea were reported to be flavonols containing catechin that are responsible for the bitterness and astringency taste (Drewnowski and Gomes-Careros, 2000) of many foods and beverages. In addition, bitter taste and astringency in DGTD tea were less than *C. sinensis* tea, which may be because the latter are rich of tannins (Asok and Upadhyaya, 2012). Boonwithawacharoen (2006) studied tannins in green tea, oolong tea, black tea and mulberry tea and found that *C. sinensis* tea had a higher capacity than the others. However, on balance the attributes of the DGTD tea were similar to those of *C. sinensis* tea but with the added advantage of the jasmine odor. Jasmine rice odor was absent in *C. sinensis* tea.

Table 4.6 Descriptors and definitions used by the ten trained panelists to describe sensory attributes of DGTD tea

Descriptors	Definition
<i>Appearance</i>	
Yellowness	The intensity of yellowness from light yellow to dark yellow
Clearness	The clearness concerned with turbidity of the samples
<i>Odor</i>	
Tea odor	The aromatics concerned with oolong tea
Hay odor	The aromatics concerned with rice straw
Green odor	The aromatics concerned with fresh grass
Seaweed odor	The aromatics concerned with natural dry seaweed (<i>Kappaphycus alvarezii</i>) from a Chinese restaurant
Jasmine rice odor	The aromatics concerned with Thai steamed Khao dawk mali 105 rice (<i>Oryza sativa</i>)
<i>Flavor</i>	
Tea flavor	The aromatic and taste concerned with oolong tea
Seaweed flavor	The aromatic and taste concerned with natural dry seaweed (<i>Kappaphycus alvarezii</i>) from a Chinese restaurant after steeping in boiled water for 3 min
<i>Taste</i>	
Bitter taste	A basic taste factor of paracetamol in water is typical
Astringent taste	The taste compared with raw cultivated banana peel

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Table 4.7 Standard references of sensory attributes and intensity scores given by ten trained panelists in the descriptive analysis of DGTD tea and *C. sinensis* tea bag from Japan

Attributes	Reference standards	Intensity reference standard	Intensity DGTD (SD)	Intensity <i>C. sinensis</i> (SD)
Appearance				
Yellowness	- Munsell book scales	-	6.5 (0.68)	6.3 (0.47)
	- 5Y8/2	0	-	-
	- 5Y8/4	7.5	-	-
	- 5Y8/6	15	-	-
Clearness	- C29 Distilled water	0	8.5 (0.64)	8.1 (0.55)
	- Wheat flour solution 0.01g: 100mL water	13	-	-
Odor				
Tea odor	- Oolong tea bag steeping 3 min in 150 mL water (98°C)	10	3.2 (0.67)	6.7 (0.58)
Hay odor	- Dry rice straw	13	0.68 (0.33)	0
Green odor	- Fresh grass	7	2.5(0.56)	0.7 (0.33)
Seaweed odor	- Natural dry 'Sakol' seaweed (<i>Kappaphycu salvarezii</i>)	15	12.7 (0.84)	10.9 (0.91)
Jasmine rice odor	- 30 g of cooked Khao dawk mali 105 rice (<i>Oryza sativa</i>) warm at 70°C	9	3.7 (0.90)	0
Flavor				
Tea flavor	- Oolong tea bag steeping 3 min in 150 mL water (98°C)	10	2.4 (0.61)	5.6 (0.68)
Seaweed flavor	- Natural dry 'Sakol' seaweed (<i>Kappaphycu salvarezii</i>) for 5 g steeped for 3 min in boiled water	15	7.5 (0.89)	8.2(0.75)
Taste				
Bitter taste	- Paracetamol solution 0.5g: 100 mL water	10	3 (0.72)	7 (0.30)
Astringent taste	- Cultivated banana peel (2 g of totally green banana peel)	7.5	0.7 (0.43)	6.4 (0.58)

Note: Values in parenthesis were standard deviation (SD) of panels mean scores.

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4.5 Identification volatile compound in DGTD tea

The GC-MS chromatography of the odor profile of DGTD tea (Figure 4.6) was identified 48 volatile compounds and grouped in classes of substances which could be described approximately by library from mass spectrometer until using external standard and GC-O the volatile compounds could be described accurately. Including 16 hydrocarbons, 9 aldehydes, 7 acids, 7 esters, 6 ketones, 2 Aromatic organic compound, 2 alcohols and 1 phenolic compound as shown in Table 4.8.

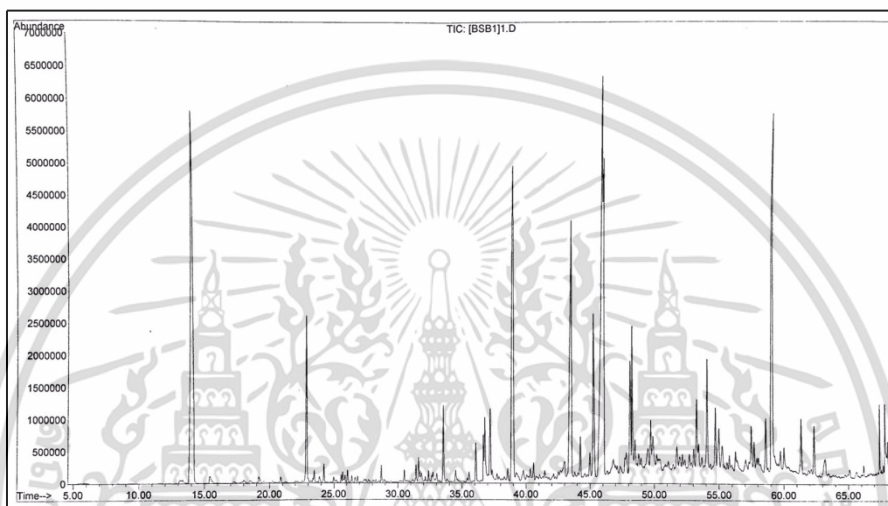


Figure 4.6 The chromatography of the odor profile of DGTD tea

Hydrocarbons were the major group, detected 14 identified, this group including cyclododecane, pentadecane, pentacosane, hexadecane, cyclotetradecane, heptadecane, octadecane, 1-nonadecane, cyclopenta [g] -2-benzopyran, 1-heptadecane, hexahydro plotaxene, tetracosane, heneicosane, especially naphthalene and cyclohexadecane, which provided floral, fruity and grassy odor. In a group of aldehyde, there were 9 aldehyde compounds including nonanal which provided grassy, tea and vegetable odor (Moon *et al.*, 2006), decanal provided herbal, fatty, orange peel and floral (Lv *et al.*, 2012), 1-cyclohexene-1-carbox aldehyde, 2,6,6-trimethyl referred to sweet, orange, lime and citrus (Schuh and Schieberle, 2006), vanillin was detected and provided floral, vanilla and sweet (Schuh and Schieberle, 2006). Dodecanal provided beefy and citrus (Liu *et al.*, 2018), tetradecanal, octanal, hexadecanal and benzyl (dideuterated) methyl ether were also detected as aldehyde compounds. Seven acid compounds such as Methyl salicylate showed sweet and minty (Wang *et al.*, 2008), dodecanoic acid, lauric acid which provided cheese odor (Poveda *et al.*, 2008), 1,2-benzenedicarboxylic acid provided cake and grass (Liu *et*

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al., 2018), n-hexyl salicylate showed sweet, herbal, floral, jasmine and orchid (Luebke, 1987), hexadecanoic acid, palmitic acid provided fruit and sour (Liu *et al.*, 2018). Moreover, tetradecanoic acid, myristic acid, and 2-propenoic acid, 3-(4-methoxy)-, were also found in this area. A total of 7 ester compounds identified butanoic butyl ester provided fruity and pineapple odor (Lv *et al.*, 2012). Benzoic acid, ethyl ester, ethyl benzoate which provided sweet, wintergreen, fruity (Mosciano, 1991), 2-butenedioic acid, dibutyl ester provided faint odor (O'Neil, 2013), isopropyl myristate provided faint oily fatty, benzoic acid, phenylmethyl ester provided fruity odor (Liu *et al.*, 2018), homomenthyl salicylate provided mild menthol (Luebke, 1986). This group was also including isopropyl palmitate provided bland oily (U.S. National Library of Medicine, Nation Institutes of Health, 2019). Six ketone compounds were identified; the first one was acetophenone provided resembling the odor of oranges, sweet and almond, (Kadow *et al.*, 2013), bicyclo (2.2.1) heptan-2-one showed fragrant and penetrating odor (U.S. National Library of Medicine, Nation Institutes of Health, 2019), (E)-6,10-dimethyl-5,9-undecadien-2-one provided fresh, floral, rose, green, fruity (Luebke, 1985), benzophenone provided rose-like (U.S. National Library of Medicine, Nation Institutes of Health, 2019) and there were ethanone and 3-buten-2-one, also found in this group. Alcohol compounds providing freshly cut grass, green by 1-hexanol, 2-ethyl- (Farina *et al.*, 2015) and ethanol provided pleasant odor and strong alcoholic (Richard and Lewis, 2012; U.S. National Library of Medicine, Nation Institutes of Health, 2019; O'Neil, 2013). There were only 2 aromatic organic compounds were found; benzothiophene provided flowery odor, sweet, rose-like and 2(4H)-benzofuranone provided tea-like odor (U.S. National Library of Medicine, Nation Institutes of Health, 2019). Phenol was in phenolic compound group, it provided sweet odor (Zhang *et al.*, 2010).

Volatile compounds detected in DGTD tea were previously reported by Baldermann *et al.* (2014) who used HS-SPME/GC-MS and found about 38 volatile compounds in high grade tea products including green tea, oolong tea and black tea, found that some of these volatile compounds were similar to volatile compounds of DGTD tea. These were 1-hexanol, butanoic acid, butyl ester, methyl salicylate and ethanone but phenol was found only in oolong tea, black tea and DGTD tea. Wu *et al.* (2016) reported volatile compounds in Yunnan sun-dried Pu-erh green tea included naphthalene, methyl salicylate, tetradecane, pentadecane and hexadecane which were also found DGTD tea. Moreover Wu *et al.*

(2016) reported about aroma compounds of baked Wujiatai green tea included 1-hexanol, เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า ไม่ว่าจะกรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ตัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้

naphthalene, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, nonanal, decanal, dodecanol, methyl salicylate, and hexadecanoic acid methyl ester. These compounds were also found in DGTD tea. Shi *et al.* (2018) reported that the volatile compounds found in tea, included benzoic acid, methyl ester, methyl salicylate, hexanoic acid, hexyl ester, hexadecanoic acid, methyl ester, nonanal, decanal, dodecane, tetradecane, pentadecane and hexadecane, which were also found in DGTD tea. Tuan *et al.* (2016) reported that volatile compounds in 14 different commercial brands of black tea samples they tested were decanal, tetradecane, hexadecane, heptadecane and naphthalene which were also detected in DGTD tea. Sakulnamrat *et al.* (2018) reported volatile compounds identified 5 similar compounds in Khao Dok Mali 105 jasmine rice young leaf tea with DGTD tea; dodecane, pentadecane, tetracosane, nonadecane, heneicosane and also identified 3 similar compounds in Homnil jasmine rice young leaf tea with DGTD tea; dodecane, tetradecane, and cyclohexanone, 4-hydroxy-4-methyl. Nonetheless the exceptional of volatile compounds in DGTD tea that rarely detected in other teas was vanillin compound which provided vanilla-like and sweet odor.

There were many volatile compounds that were detected by GC-MS/HS-SPME, three of them were matched with sensory characteristics by ten trained panelists (Table 4.6 and Table 4.8). These were tea odor, green odor, and jasmine rice odor. Green odor from sensory characteristics which was referred as fresh grass and initiated by the following volatile compounds: cyclohexadecane, nonanal, dodecanoic acid, E)-6,10-dimethyl-5,9-undecadien-2-one, 1,2-benzenedicarboxylic acid and 1-hexanal, 2-ethyl- that provided green, grassy and fresh cut grass. Tea odor which was detected in oolong tea bag that had been steeped for 3 min in 150 mL water (98°C) and also initiated by Nonanal that provided tea odor. Lastly, jasmine rice odor which was detected by 30 g of cooked Khao dawk mali 105 rice in (*Oryza sativa*) warm at 70°C and originated odor from n-hexyl salicylate provided jasmine-like. Moreover, two attributes were able to be detected only by sensory characteristics. These were; hay odor and seaweed odor. However, some positive volatile compounds which provided positive odor which were identified only by GC-MS such as sweet odor, cake odor, cheese odor, vanilla, flower odor, rose-like, orchid odor, herbal, minty, fruit-like, orange odor and citrus that were not able to detect by sensory characteristics.

Table 4.8 The volatile compounds of the odor profile of DGTD tea

RT ^a	RI ^b	Compound ^c	MS ^d match value	Odor description ^e	Relative content(%) ^f
Hydrocarbons					
22.84	1176	Napthalene	91	Floral, fruity	20.26
37.22	1472	Cyclododecane	95	-	4.10
38.56	1496	Pentadecane	94	-	1.69
40.33	1527	Pentacosane	87	-	1.68
44.24	1597	Hexadecane	98	-	1.48
48.45	1673	Cyclotetradecane	94	-	0.28
48.51	1674	Cyclohexadecane	97	Grassy odor	0.32
49.73	1679	Haptadecane	97	-	5.58
54.99	1734	Octadecane	95	-	7.23
58.99	1841	1-Nonadecane	95	-	4.32
56.36	1823	Cyclopenta[g]-2-benzopyran,	97	-	0.96
58.98	1875	Cyclohexadecane	97	-	0.32
67.70	2085	1-Heptadecane	89	-	0.32
		Hexahydroplotaxene			
66.84	2054	Tetracosane	99	-	1.49
67.76	2091	Heneicosane	97	-	1.57
Aldehydes					
19.22	1102	Nonanal	86	Grassy, tea and vegetable	0.95
24.21	1203	Decanal	95	Herbal, fatty, orange peel and floral,	1.77
24.66	1213	1-Cyclohexen-1-carbox Aldehyde, 2,6,6- trimethyl	94	Sweet, orange, lime and citrus	0.28
32.73	1389	Vanillin	95	Floral, vanilla and sweet	0.22
33.54	1406	Dodecanal	98	Beefy, citrus and mint	8.08
44.90	16.08	Tetradecanal	98	-	0.23
51.71	1734	Octanal	95	Fruity	7.23
55.73	1811	Hexadecanal	90	-	0.53
61.87	1932	Benzyl methyl ether	91	-	0.43

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Table 4.8 The volatile compounds of the odor profile of DGTD tea (Cont'd)

RT ^a	RI ^b	Compound ^c	MS ^d match value	Odor description ^e	Relative content(%) ^f
Acid					
23.34	1186	Methyl salicylate	97	Sweet, spicy, minty	0.79
42.40	1564	Dodecanoic acid, Lauric acid	95	Cheese	1.52
43.55	1584	1,2-Benzenedicarboxylic acid	97	Cake, grass	55.33
48.09	1666	n-Hexyl salicylate	90	Sweet, herbal, floral, jasmine, orchid	1.54
53.21	1762	Tetradecanoic acid, Myristic acid	97	-	3.51
61.21	1919	Hexadecanoic acid, Plamitic acid	98	Fruit, sour	0.47
67.98	2095	2-Propenoic acid, 3-(4- methoxyl)-	95	-	0.28
Esters					
31.61	1364	Butanoic acid, butyl ester	87	Fruity, pineapple	2.21
39.79	1517	Benzoic acid, Ethyl ester, Ethyl benzoate	89	Sweet, wintergreen, and fruit	2.50
41.06	1540	2-Butenedioic acid, Dibutyl ester	83	Faint odor	4.15
56.23	1821	Isopropyl Myristate	96	Faint, oily and fatty	0.70
57.85	1853	Benzoic acid, Phenylmethyl ester	90	Fruity odor	0.52
58.58	1867	Homomenthyl salicylate	95	Mild menthol	2.65
66.15	2019	Isopropyl Palmitate	90	Bland oily	1.25
Ketones					
17.21	1063	Acetophenone	80	Sweet, almond, orange	0.44
21.13	1141	Bicyclo(2.2.1) heptan-2-one	95	Fragrant, penetrating odor	0.26
34.49	1423	Ethanone	93	-	1.78
35.34	1438	(E)-6,10-Dimethyl-5,9- Undecadien-2-one	97	Fresh, floral, rose, green and fruity	1.33
37.40	1475	3-Buten-2-one	83	-	0.44
45.26	1615	Benzophenone	91	Rose-like	0.51

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Table 4.8 The volatile compounds of the odor profile of DGTD tea (Cont'd)

RT ^a	RI ^b	Compound ^c	MS ^d match value	Odor description ^e	Relative content(%) ^f
Aromatic organic compounds					
23.20	1183	Benzothiophene	94	Flowery odor, sweet, rose-like	0.17
39.84	1581	2(4H)- Benzofuranone	91	tea-like odor	0.89
Alcohols					
15.40	1028	1- Hexanol, 2-ethyl-	90	Freshly cut grass, green	1.74
23.70	1193	Ethanol	90	Pleasant odor, strong alcoholic	0.57
Phenolic compound					
30.73	1345	Phenol, 2,4-bis (1,1- dimelethly)	91	Sweet, phenolic, compound	0.13

^a RT, The retention time (min) of identified volatiles compound

^b RI, retention indices as determined on HP-5MS column using a homologous series of n-alkanes (C₇-C₃₀).

^c Compounds listed according to their structure

^d MS match value of Nist Mass Spectral library

^e Odor description: odor description as perceived by panelists according to GC-O in the relevant literatures

^f The relative percentage content (%) as ratio each peak area

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4.6 Effect of storage time on physical property, bioactive compound, biological quality and sensory acceptance of DGTD tea

4.6.1 Physical properties

Physical properties of stored DGTD tea for 0, 4, 8, 10 and 12 months were studied through the analysis of moisture content, water activity and color values in CIE system; L^* , a^* and b^* and ΔE . Table 4.9 show that the samples, which were stored for 10 and 12 months in aluminum foil bags, had moisture contents that tended to significantly ($p < 0.05$) increase with time. The moisture content of 12 month storage samples increased from 2.43% to 3.08%, which was the moisture content before storage. The dried DGTD tea product contained rather low moisture content, which was lower than the moisture content of the product and show that it could easily absorbed moisture back into the product (Jamali *et al.* 2006). In general moisture content of dried tea leaves was reported to be about 3-5% (Temple and Boxtel, 1999; Winyangkul *et al.*, 2007). Moreover, the moisture content was related to A_w which presented a small non-significantly ($p \geq 0.05$) increase. The determination of color values showed that the 12 month storage period was not significantly different in L^* (lightness), a^* (redness) and ΔE while b^* (yellowness) significantly ($p < 0.05$) resulting in more yellowish color of the rice tea product. Ostadalova *et al.* (2015) studied green tea quality after storage in 6 different conditions. Despite the different conditions, all of the samples had similar degrees of decreasing chlorophyll levels after prolonged storage. As a natural pigment in tea leaves, when chlorophyll decreased the green tea color may change as well. Pinghai (2006) confirmed that the access of oxygen to tea samples affected the degradation of natural pigments, including chlorophyll, which may be identical. When exposed to oxygen magnesium may be released from the chlorophyll molecule and be replaced by hydrogen which leads to the creation of pheophytins, the hydrogen derivatives, and then pyro pheophytins and pheophorbides. These activities can effectively change the color of the tea from green to brown; an effect that was also described by Mayer (1987).

Table 4.9 Moisture contents (%), water activity (A_w), L^* , a^* , b^* and ΔE of DGTD tea at 0, 4, 8, 10 and 12 months storage time.

Storage (month)	Moisture contents (%)	A_w^{ns}	L^{*ns}	a^{*ns}	b^*	ΔE^{ns}
0	2.43 ^b ±0.22	0.33±0.00	47.37±1.02	-5.78±0.33	15.74 ^b ±0.46	0
4	2.44 ^b ±0.12	0.35±0.00	47.71±1.15	-6.15±0.06	15.88 ^b ±0.71	1.20±0.27
8	2.45 ^b ±0.19	0.34±0.01	48.06±0.45	-5.92±0.43	15.98 ^b ±0.47	0.83±0.63
10	2.91 ^a ±0.09	0.36±0.01	49.86±1.69	-6.33±0.21	17.19 ^{ab} ±0.73	2.96±1.80
12	3.08 ^a ±0.04	0.36±0.02	50.29±2.93	-6.46±0.63	17.50 ^a ±1.13	3.63±2.94

Note: ^{a,b} Mean±SD, the different small letter in each column was significantly different at $p < 0.05$.

^{ns} Mean±SD, the ns letter in each column was non-significantly different at $p \geq 0.05$.

4.6.2 Bioactive compounds

The DGTD tea samples, stored for 0, 4, 8, 10 and 12 months, were analyzed for total phenolic content (Figure 4.7), antioxidant activities by FRAP, DPPH and ABTS (Figure 4.8). Total phenolic contents of stored DGTD tea tended to decrease significantly ($p < 0.05$) when stored for 10 and 12 months. The values were 9.53, 9.9, 9.78, 8.96 and 8.53 mg GAE/g sample for 0, 4, 8, 10 and 12 months, respectively. The antioxidant activity, as measured by FRAP, were decreasing significantly during storage for 4, 8, 10 and 12 months; 268.57, 227.83, 226.64 and 225.45 mM TE/g sample, respectively. The antioxidant activity, as measured by DPPH and ABTS, were also decreasing significantly after 12 months of storage; 34.08, 33.46, 29.56, 29.4 and 27.77, and 108.89, 118.22, 108.67, 107.56 and 104.89 mM TE/g sample, respectively. The decrease in total phenolic content corresponded with decreasing of antioxidant activity. It might roughly be assumed that phenolic compounds were the main phytochemical components holding the product's antioxidant activity. Mirasoli *et al.* (2014) studied quality changes in commercial green tea leaves during a long-term storage and found that tea leaves stored for two years had less of polyphenols.

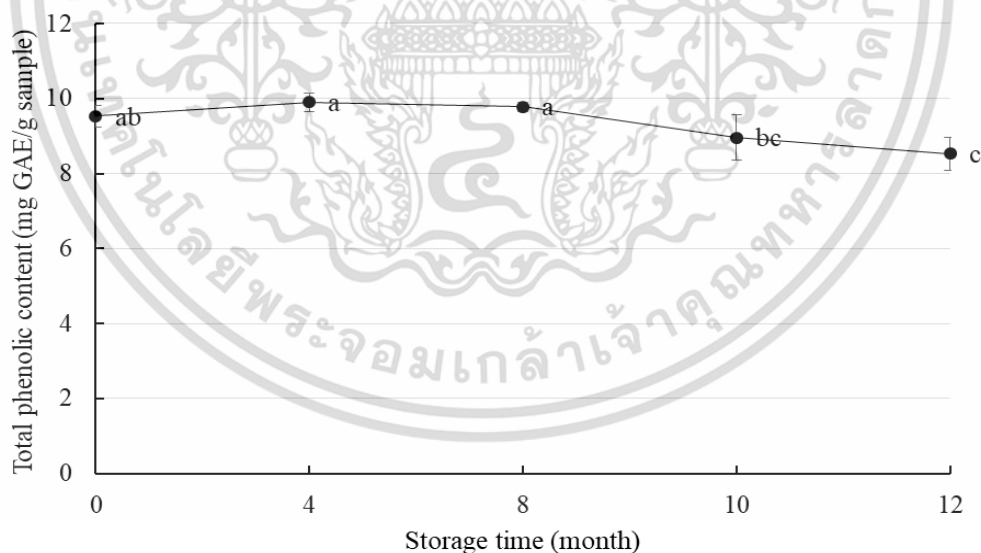


Figure 4.7 Total phenolic content (mg GAE/g sample), in DGTD tea stored at 0, 4, 8, 10 and 12 months

Note: ^{a,b,c}, the different small letter in each line was significant different at $p < 0.05$

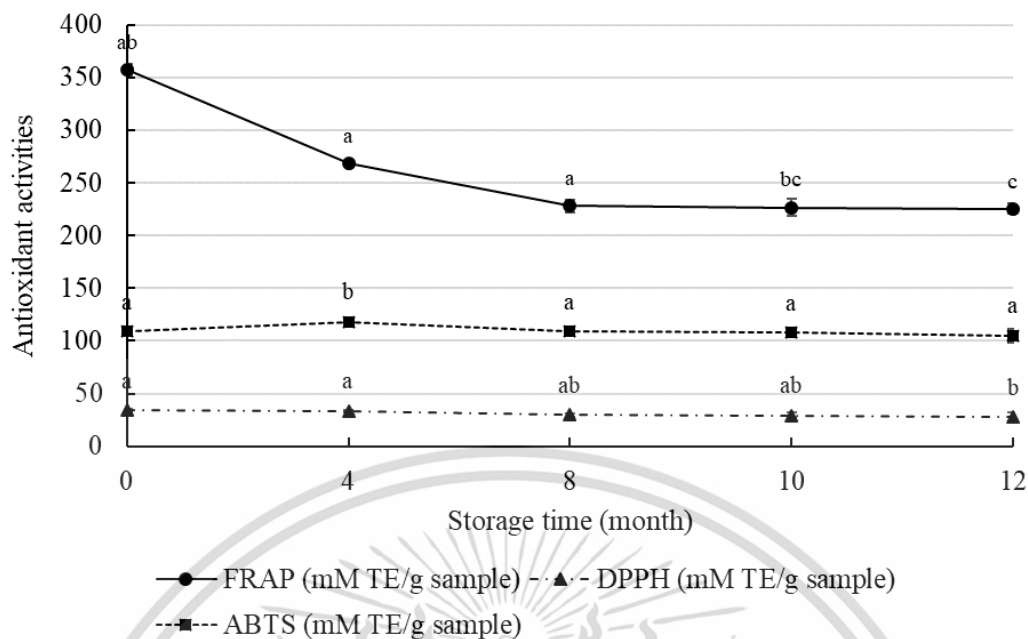


Figure 4.8 Antioxidant activities by FRAP, DPPH and ABTS (mM TE/g sample) in DGTD tea stored at 0, 4, 8, 10 and 12 months

Note: ^{a,b,c}, the different small letter in line was significant different at $p \leq 0.05$.

4.6.3 Microorganism (Aerobic Plate Count, Yeast and Mold)

Aerobic plate count, yeast and mold in DGTD tea samples kept for 12 months was shown in Table 4.9, which shows the levels of microorganism from total plate count analysis, increased continuously from 4, 8, 10 and 12 months of storage; 9.7×10^2 , 3.3×10^3 , 9.3×10^3 and 1.94×10^4 cfu/g, respectively. No yeast was found in any sample during the storage, meanwhile mold was found when the sample were stored for 10 and 12 months; 1×10^1 cfu/g and 2×10^1 cfu/g, respectively. The Office of Industrial Product Standards, Ministry of industry (2015) launched Thai community product standard Tea (2015) indicating that the amount of total microorganism in tea products must be less than 1×10^4 cfu/g. In DGTD tea found that the amount of total microorganism was over the standard after 10 months storage and also for mold (1×10^1 cfu/g) in the 10 month of storage. Therefore the microbial quality of the tea samples did not conform to the standard. The rationale of the significant amount of microorganism present in DGTD tea product might be due to contamination from the soil in which the plants were grown. The contamination could be from the cutting of the plants only 1 cm above soil level. This result was different from *Camellia sinensis* tea leaves where the raw material is from two terminal leaves plus one bud, which reduces

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or eliminates the chances of dirt contamination. Meeting the standard requirement, to reduce the initial microbes in raw DGTD rice grass leaves is essential, for instant, carefully washing of the cut DGTD rice grass leaves or applying organic disinfectant e.g. acetic acid at a low concentration and thoroughly re-washing again with tap water to avoid acidic flavor.

Table 4.10 Total plate count, yeast and mold in stored DGTD tea

Storage (month)	Total plate count (cfu/g)	Yeast/Mold (cfu/g)
0	3.6×10^2	ND
4	9.7×10^2	ND
8	3.3×10^3	ND
10	9.3×10^3	1×10^1
12	1.94×10^4	2×10^1

Note: ND, the ND letter was non detected.

4.6.4 Sensory evaluation

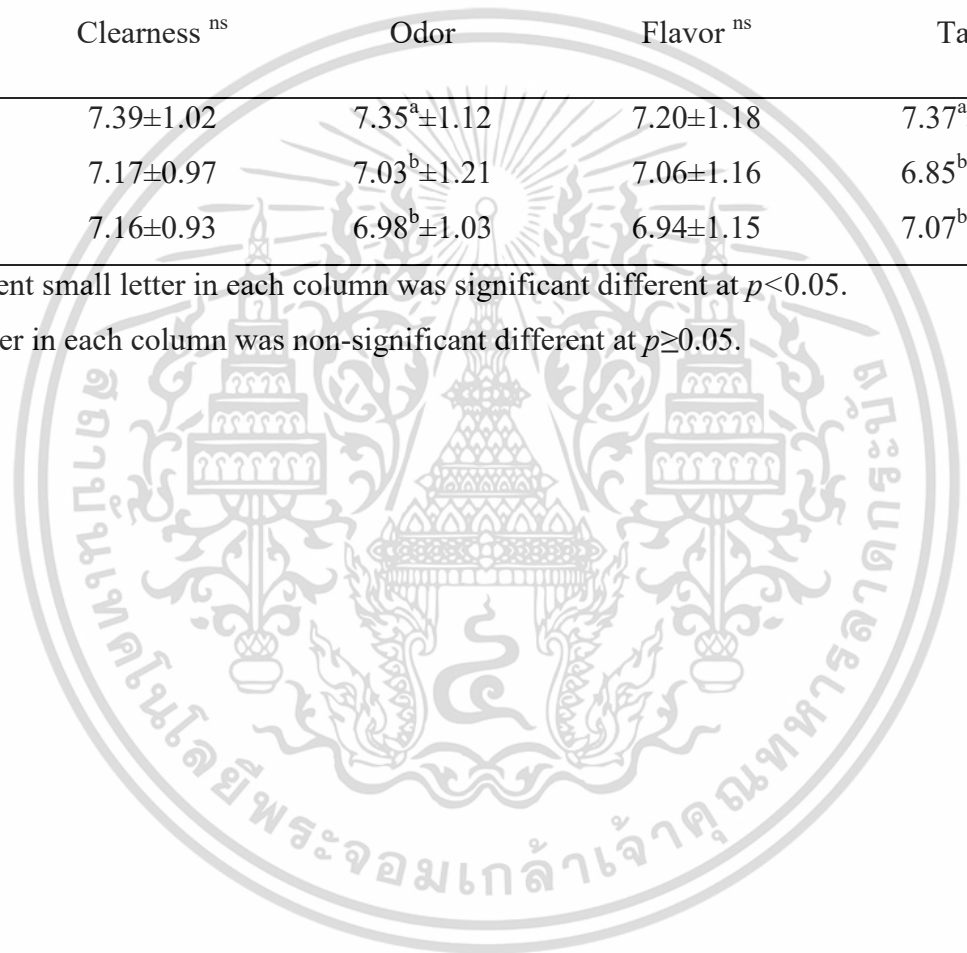
A nine-point hedonic scale sensory test was used to evaluate DGTD tea after it had been stored for 0, 4 or 8 months. The parameters tested were color, clearness, odor, flavor, taste and overall liking, results were shown in Table 4.10. Results showed the color and clearness scores after 8 months of storage were not significant different ($p \geq 0.05$). For the odor and taste attributes, the scores were significantly ($p < 0.05$) decreased, which started from the 4 month of storage. However, after 8 months storage there were no significant effects on flavor and overall liking scores of rice tea. Lee and Chamber (2010) studied the flavor characteristic of Korean green tea packaged in metalized multilayer polyethylene films and stored at an ambient temperature of $\sim 20^\circ\text{C}$. They found that the flavor and aroma of green tea changed when stored for over two years after packing.

Table 4.11 Sensory evaluation of stored DGTD tea at 0, 4 and 8 months

Storage (month)	Color ^{ns}	Clearness ^{ns}	Odor	Flavor ^{ns}	Taste	Overall liking ^{ns}
0	6.92±1.14	7.39±1.02	7.35 ^a ±1.12	7.20±1.18	7.37 ^a ±1.16	7.30±1.14
4	6.69±0.98	7.17±0.97	7.03 ^b ±1.21	7.06±1.16	6.85 ^b ±1.09	7.09±0.98
8	6.89±0.88	7.16±0.93	6.98 ^b ±1.03	6.94±1.15	7.07 ^b ±1.09	7.12±1.02

Note: ^{a,b} Mean±SD, the different small letter in each column was significant different at $p < 0.05$.

^{ns} Mean±SD, the ns letter in each column was non-significant different at $p \geq 0.05$.



CHAPTER 5

CONCLUSIONS

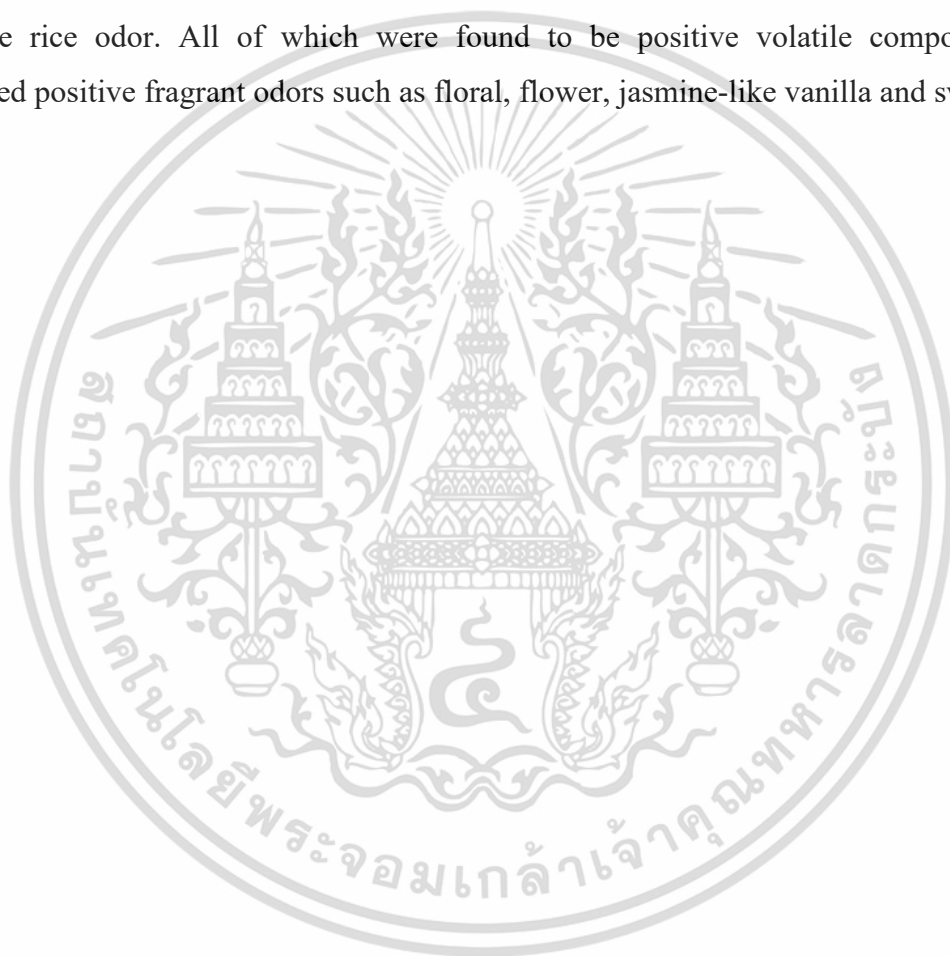
1. Damgatondam (DGTD) upland rice grass leaves, which had been sown one month earlier, were successfully made into green tea by steaming, pan roasting and tray drying. From the experiment it was possible to define exactly the optimum processing conditions in this experiment, which were steaming at 98°C for 60 sec, pan roasting at 70°C for 30 min and tray drying in an electrical tray dryer at 60°C for 90 min. In terms of chemical composition and antioxidant activity, it was found that DGTD tea was acceptable and its composition comparable to green tea made from *C. sinensis*. The sensory characteristics, from descriptive analysis of the product, showed 11 particular attributes consisting of yellowness, clearness, tea odor, hay odor, green odor, seaweed odor, jasmine rice odor, tea flavor, seaweed flavor, bitter taste and astringent taste and its jasmine odor was positively accepted.

2. Brewing DGTD tea weight 1, 1.5 and 2 g at temperature 80, 90 and 98°C affected color, bioactive compounds and sensory acceptability. The increasing of DGTD tea weight and temperature resulting in darker color, greener, yellower, and brighter of tea, increasing in bioactive compounds in terms of total phenolic content and antioxidants by FRAP, DPPH and ABTS become the highest when brewing 2g of DGTD tea at 98°C (18.27 mgGAE/g, 114.40, 47.14 and 55.44 mM TE/g, respectively). In addition, sensory evaluation in color, clearness, odor, flavor, taste and overall liking found that brewing 2 g of DGTD tea at 98°C was effective the highest score in all attributes including color was 7.24, clearness was 7.67, odor was 7.52, flavor was 7.65, taste was 7.82 and overall liking was 7.79 respectively.

3. Storage of DGTD tea packed in paper tea bags, sealed in laminated aluminum plastic bags and stored at room temperature of ~30°C retained their quality for up to 8 months. The possibility of having an alternative tea beverage was established, but the methods to minimize their contamination from microbes requires more research. Tea processing and packaging are the two factors that could be modified in order to extend the product shelf-life. Bioactive compounds (TPC, FRAP, DPPH and ABTS) decreased significantly ($p < 0.05$) over the storage time. The sensory evaluation panel detected significant ($p < 0.05$) reductions in odor and taste between the freshly processed and those that had been stored

for 4 months, but there was no further reduction after longer storage. There were no significant ($p \geq 0.05$) differences in color, flavor, and overall liking perceived by the panel.

4. Identification of volatile compounds in DGTD tea using headspace-solid phase micro-extraction (HS-SPME) found 48 volatile compounds that were identified and grouped into classes of substances, including hydrocarbons (29.16%), aldehydes (18.78%), acids (14.58%), esters (14.58%), ketones (12.50%), aromatic organic compounds (4.16%), alcohols (4.16%), and phenolic compound (2.08%). Three of them were matched with sensory characteristics by ten trained panelists. These were tea odor, green odor, and jasmine rice odor. All of which were found to be positive volatile compounds that provided positive fragrant odors such as floral, flower, jasmine-like vanilla and sweet odor.



เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
ไม่ว่ากรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ดัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้

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เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า ไม่ว่าจะกรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ตัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้

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เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
ไม่ว่ากรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ตัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้



Appendixes

เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
ไม่ว่ากรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ดัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้



Appendix A

Steps of DGTd tea process

เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
ไม่ว่ากรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ดัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้



1. Fresh young rice grass



5. Withering 5 min



2. Cutting 1 cm in pieces



6. Pan roasting for 30 min



3. Put in a single rack steamer



7. Tray drying for 90 min



4. Steaming for 60 sec



8. Grinding and packing

เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
ไม่ว่ากรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ตัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้



Appendix B

Statistical analysis

เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
ไม่ว่ากรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ดัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้

Appendix B: Moisture content of DGTD rice leaves pan roasted (PR) for 5, 10, 15, 20, 25 or 30 min prior to dehydration in an electrical cabinet tray dryer (TD) at 60°C for 30, 45,60 or 90 min

Source	SS	Df	MS	F	Sig.
Corrected Model	124.480 ^a	23	5.412	41.785	.000
Intercept	1664.068	1	1664.068	12847.466	.000
Time (TD)	58.495	3	19.498	150.537	.000
Time (PR)	61.441	5	12.288	94.871	.000
Time(TD) * Time(PR)	4.544	15	.303	2.339	.013
Error	6.217	48	.130		
Total	1794.765	72			
Corrected Total	130.697	71			

Note: a. R Squared = .952 (Adjusted R Squared = .930)

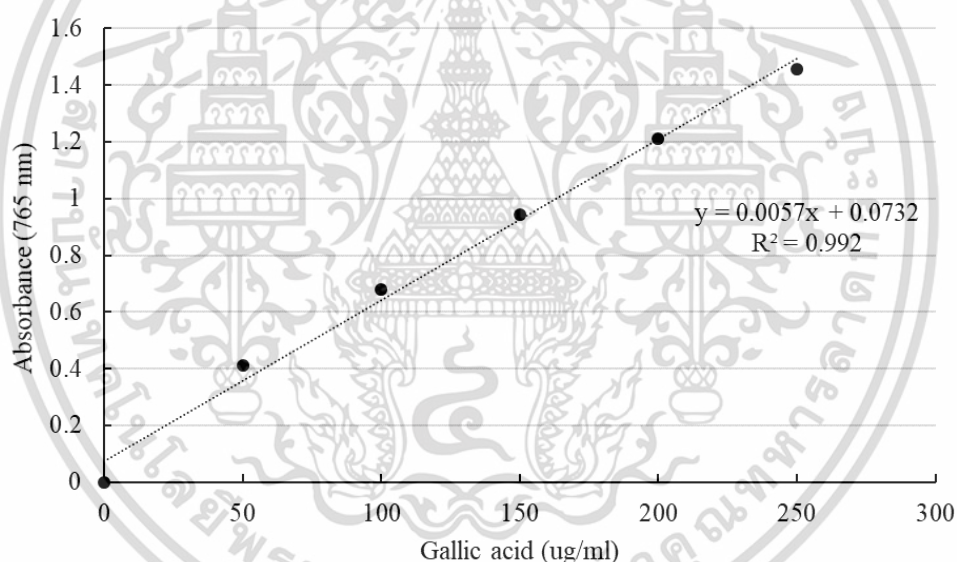
PR = Pan roasted, TD = Tray dried



เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า
ไม่ว่ากรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ดัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้

Appendix C1: Standard curve of Total Phenolic Content assay

The stock Gallic acid solution was prepared by dissolving 0.025 g in distilled water and completed to volume 100 ml. It was then keep in dark place by wrapping with aluminum foil. 0, 100, 200, 300, 400, and 500 μ l of stock Gallic acid solution was placed in test tubes which wrapped by aluminum foil. Then, distilled water was added to each tube to bring the total volume of 500 μ l. The final mass of Gallic acid in each test tube was 0, 50, 100, 150, 200 and 250 μ g. Added 5 ml of Folin-Ciocalteu (1:10, Folin-Ciocalteu: distilled water), shook well and waited for 5 minutes. Thereafter, the reaction was neutralized by 4 ml of 1M Na_2CO_3 (10.6 g Na_2CO_3 dissolve in 100 ml distilled water) for 10 minutes. The absorbance was read at 765 nm. 50:50 (water/methanol) was used as blank. The standard curve was observed:



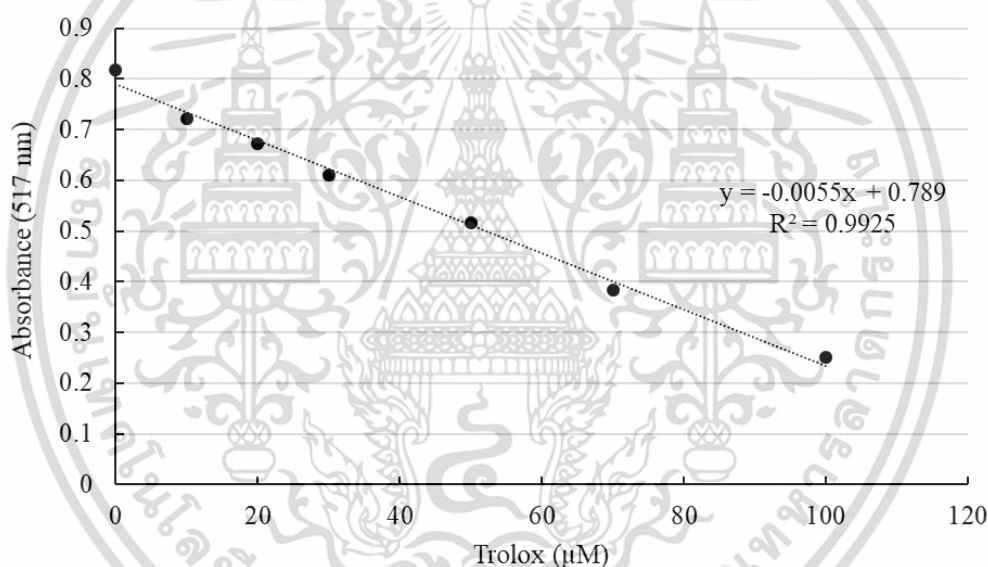
Where y: Absorbance
 x: Mass of Gallic acid (μg)

เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า ไม่ว่าจะกรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ดัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้

Appendix C2: Standard curve of DPPH assay and calculation

The stock Trolox solution was prepared by dissolving 0.025g of Trolox in methanol and completed to volume 100 ml. Took 0 (control), 15, 30, 45, 75, 105 and 150 μl of stock Trolox solution to each test tube which wrapped by aluminum foil. Add water in each test tube to get the final volume of 1500 μl . The observed Trolox concentration in each test tube was 10, 20, 30, 50, 70 and 100 $\mu\text{mol/L}$ (μM).

Thereafter, add 1.5 ml DPPH working solution in each test tube, shook well and allowed the reaction for 30 minutes. Methanol was used as blank. The absorbance was then read at 517 nm. The standard curve was plotted as the following:



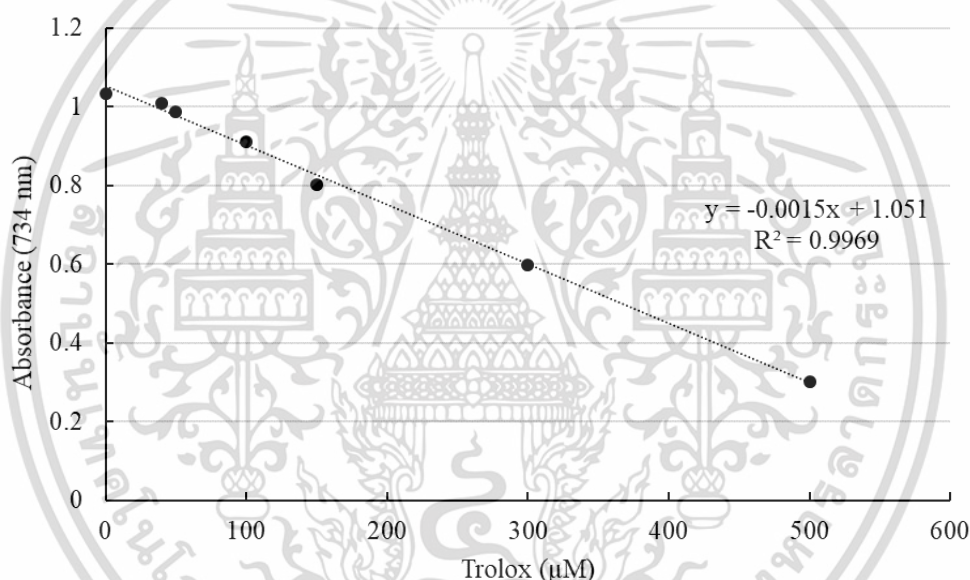
Where y: Inhibition
x: Trolox concentration

เอกสารนี้เป็นเอกสารที่สงวนไว้สำหรับการใช้งานเพื่อการศึกษาเท่านั้น ไม่อนุญาตให้นำไปใช้ประโยชน์ด้านการค้า ไม่ว่าจะกรณีใดๆ ทั้งสิ้น อีกทั้งห้ามมิให้ตัดแปลงเนื้อหา และต้องอ้างอิงถึงเจ้าของเอกสารทุกครั้งที่มีการนำไปใช้

Appendix C3: Standard curve of ABTS assay and calculation

The stock Trolox solution was prepared by dissolving 0.025g of Trolox in methanol and completed to volume 100 ml. Took 0 (control), 6, 7.5, 15, 22.5, 45 and 75 μl of stock Trolox solution to each test tube which wrapped by aluminum foil. Add water in each test tube to get the final volume of 150 μl . The observed Trolox concentration in each test tube was 40, 50, 100, 150, 300 and 500 $\mu\text{mol/L}$ (μM).

ABTS working solution 2.85 ml was allowed to react in each test tube contained Trolox solution. After 2 hours incubation in dark place, the solution was read absorbance at 734 nm. Methanol was used as blank. The standard curve was observed below:

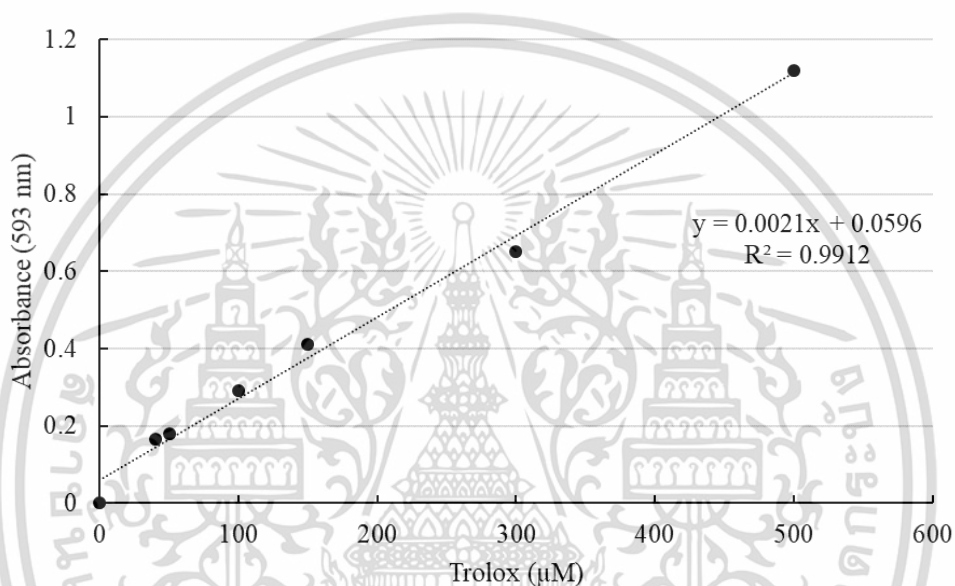


Where y: Inhibition
x: Trolox concentration

Appendix C4: Standard curve of FRAP assay and calculation

The same in Appendix A2, for FRAP assay, the Trolox concentrations were from 40 to 500 μM .

The FRAP working solution 2.85 ml was allowed to react with Trolox in each test tube for 30 minutes and then the absorbance was read at 593 nm. Methanol was used as blank. The standard curve was plotted as below:



Where y: Absorbance
x: Trolox concentration

AUTHOR BIOGRAPHY



Narissara Uthai grew up in Bangkok, Thailand and received her Bachelor's degree in Food and Nutrition in 2005 from Rajamangala University of Technology Krungthep and Master's degree of Science in Food catering in 2008 from King Mongkut's Institute of Technology Ladkrabang. She completed her Doctoral degree in Food Science in 2019 from King Mongkut's Institute of Technology Ladkrabang, where she studied processing, proximate composition, mineral content, brewing method, bioactive compound and antioxidant activity, shelf life and identification of volatile compound by using gas chromatography of rice grass tea, under the supervision of Assistant Professor Dr. Soraya Kerdpi boon and Assistant Professor Dr. Porjai Thamakorn at the Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. She currently works as a lecturer at Rajamangala University of Technology Krungthep.

E-mail address: uthai.narissara@gmail.com

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