THE EFFECT OF TEA AND SPENT TEA TOWARDS DIGESTION PROPERTIES AND DEVELOPMENT OF LOW GLYCEMIC INDEX BISCUIT

by

NURUL NADIAH BINTI ISMAIL

**Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science**

October 2018

ACKNOWLEDGEMENT

First and foremost, I would like to express my sincere gratitude and appreciation to my supervisor, Dr. Uthumporn Utra @ Sapina Abdullah, Associate Professor Dr. Tajul Aris bin Yang, and Associate Professor Dr. Fazilah binti Ariffin for their close supervision, expertise, time, patience and continuous support throughout my study and research project. . Through their supervision I have developed a set of invaluable skills that will greatly assist me in my future endeavours.

In addition, my gratitude is expanded to the wonderful groups of laboratory assistants, Master's and PhD students, and staff of School of Industrial Technology, Universiti Sains Malaysia, Pulau Pinang for their kind assistance through the entire process of completing this project. My appreciations are also extended to Professor Wan Rosli bin Wan Ishak, Mr. S. Daniel Robert, and Dr. Wan Amir Nizam bin Wan Ahmad from School of Nutrition, Universiti Sains Malaysia, Kelantan for their sincere help and guidance. Moreover, my deepest gratitude goes to my friend, Noor Diana binti Abu Bakar, master student of Professor Wan Rosli, who assisted me at Universiti Sains Malaysia, Kelantan.

Not forgetting, my friends for their suggestions, advice and moral support which stimulated me to think more critically. My special appreciations to my family members for their moral and spiritual guidance, love and encouragements in my life. Furthermore, my sincere gratitude to my husband, and everyone who directly and indirectly advised me throughout this project. Last but not least, thank you Allah, for everything.

Nurul Nadiah binti Ismail

OCTOBER 2018

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AACC	American Association of Cereal Chemist
BMI	Body Mass Index
BT	Black tea
C	Catechin
CI	Confidence of variation
cm	centimeter
CTC	Crush, Tear and Curl
CV	Coefficient of variation
DF	Dietary fiber
DM	Diabetes Mellitus
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC	(-)-epicatechin
ECG	(-)-epicatechin gallate
EGC	(-)-epigallo-catechin
EGCG	(-)-epigallocatechin gallate
$FeCl_3.6H_2O$	ferum cloride
g	gram
GAE	gallic acid equivalent
GCG	(+)-gallocatechin gallate,
GI	Glycemic index
GL	Glycemic load

GT	Green tea
HbA1C	Glycated haemoglobin
HCl	Concentrated hydrochloric acid
HDL	High Density Lipoprotein
HPLC	High Performance Liquid Chromatography
iAUC	Incremental area under curve
kg	kilogram
L	liter
LDL	Low Density Lipoprotein
mg	milligram
ml	milliliter
mM	milimole
NaOH	Sodium hydroxide
nm	nanometer
OT	Oolong tea
SD	Standard deviation
SEM	Standard error of mean
TFC	Total flavonoid content
TPC	Total phenolic content
TPTZ	2,4,6-tris(2-pyridyl)-5-triazine
v/v	volume/volume
w/v	weight/volume
WHO	World Health Organisation

μl	microliter

KESAN TEH DAN HAMPAS TEH TERHADAP SIFAT PENERIMAAN DAN PEMBUATAN BISKUT YANG RENDAH INDEKS GLISEMIK

ABSTRAK

Teh (*Camellia sinensis L.*) adalah salah satu tumbuhan yang kaya dengan kompaun fenolic and tinggi dalam kandungan antioksida serta dikenali umum untuk memberi kesihatan yang baik kepada manusia sejagat. Walau bagaimanapun, maklumat mengenai ciri-ciri kimia teh dan hampas teh sebagai hasil buangan dari kilang perindustrian masih belum mencukupi. Oleh itu, kajian ini telah mengkaji hubungan antara kesan kurangnya kandungan gula dalam darah dengan aktiviti antioksida yang terdapat dalam daun teh. Di samping itu, daun teh juga boleh digunakan sebagai bahan dalam pembuatan produk makanan. Kajian ini telah dibahagikan kepada 3 fasa. Pada fasa pertama, objektif kajian adalah untuk membanding kesan pengektrakan antara air panas dengan 50% kepekatan ethanol. Selain itu, kajian berkaitan hubungan antara sebatian kimia dalam beberapa jenis teh, seperti teh hijau, teh oolong, dan teh hitam; berserta hampas masing-masing, juga telah dilakukan. Beberapa komponen kimia seperti asid gallik, kafein, katechin, gallokatechin gallate, dan epigallocatechin, dianalisa menggunakan kaedah Kromatografi Cecair Berprestasi Tinggi. Dalam kajian ini, teh hijau (pengektrakan ethanol) didapati mengandungi jumlah fenolik and flavonoid, serta antioksida aktiviti yang tinggi berbanding teh oolong dan teh hitam. Dalam teh hitam terdapat kandungan fenolik yang lebih dikenali sebagai theaflavin. Pada fasa kedua, kajian mengenai potensi kurangnya kandungan gula dalam darah oleh 3 jenis teh dan hampas dilakukan menggunakan kaedah *in vitro*. Keputusan menunjuk bahawa semua jenis teh mempunyai kesan pengurangan kandungan gula sebagai penunjuk daripada kesinambungan kajian penyerapan kandungan gula, perenjatan kadar penyebaran gula, dan sistem model kinetik amilase dengan kadar urutan: teh hitam > hampas teh hitam, teh oolong > hampas teh oolong, teh hijau > hampas teh hijau. Pada fasa terakhir, kajian deria rasa dan kadar indeks glisemik telah dilakukan ke atas badan manusia. Berdasarkan keputusan yang direkod pada fasa kedua, teh hitam dan hampasnya telah dipilih untuk membuat biskut dengan indeks glisemik yang rendah. Teh hitam dan hampas teh yang dipilih untuk ditambah dalam kandungan biskut adalah sebanyak 0, 5, 10 dan 15%. Penerimaan deria menunjukkan skor purata penerimaan untuk kawalan, 10% teh hitam, dan 10% hampas teh hitam yang ditambah dalam biskut telah dipilih untuk kajian indeks glisemik. Keputusan menunjuk bahawa indeks glisemik biskut yang mengandungi 10% teh hitam adalah 40±5 % manakala 10% SBT adalah 46±3 %, lebih rendah berbanding dengan biskut kawalan (57±4 %). Kesimpulannya, kadar penurunan indeks glisemik boleh dicapai dengan penambahan teh hitam dan hampas teh hitam.

THE EFFECT OF TEA AND SPENT TEA TOWARDS DIGESTION PROPERTIES AND DEVELOPMENT OF LOW GLYCEMIC INDEX BISCUIT

ABSTRACT

Tea (*Camellia sinensis L.*) is one of native plants which is rich in phenolic compounds and antioxidant elements, widely recognized for its benefits for human consumption. However, the information on chemical characteristics of the tea and residual tea leaves originated from the by-product of tea beverage manufactures is still insufficient. Therefore, this study aimed to evaluate the hypoglycemic effect of antioxidant activity which is present in tea leaves, as one of food ingredients in production of low glycemic food product. This study was divided into 3 phases, while the first phase was aimed to compare the effect of different extraction conditions (hot water and 50% ethanol concentration) in order to determine the chemical compounds which are present in different types of tea (black tea (BT), oolong tea (OT), green tea (GT), spent black tea (SBT), spent oolong tea (SOT) and spent green tea (SGT)). In order to execute this goal, Gallic acid, caffeine, catechin (C), gallocatechin gallate (GCG), epicathechin (EC) and epigallocatechin (EGC) were analyzed through High Performance Liquid Chromatography (HPLC). HPLC results indicated that GT mainly consist of high amount of catechin. However, BT mainly consisted of theaflavin, since BT undergoes full fermentation. The second phase examined the hypoglycemic potential of different types of tea and spent tea leaves by means of suitable *in vitro* methods. The obtained results suggested that all types of teas had hypoglycemic effect, as indicated by significant glucose adsorption capacity, glucose diffusion retardation, and amylolysis kinetics model systems, as in trend: BT > SBT, determination were conducted on human. According to the obtained results in phase 2, BT and SBT were selected for development of low GI biscuit. Hence, BT and SBT were incorporated into biscuit formulations at 0, 5, 10 and 15%. Sensory acceptability presented that overall acceptability score for control and 10% BT biscuits were the highest among all formulation. Thus, control, 10% BT and 10% SBT of biscuits were selected for GI testing. It was observed that the GI value of biscuits with 10% BT (40±5 %) and 10% SBT (46±3 %) were significantly lower than control biscuits (57±4%). It was concluded that low GI biscuits can be produced by incorporation of BT and SBT.

CHAPTER 1

INTRODUCTION

1.1 Background

Tea (*Camellia sinensis*) is a popular beverage due to its phenomenal taste and outstanding benefits towards health (Khokhar and Magnusdottir, 2002). Moreover, tea is known to provide health benefits, especially green tea, since the beginning of its history. Generally, there are about six types of tea, while green tea, black tea, and oolong tea are the most commonly found in the market. Those teas are classified according to the steps involved during processing, known as withering, rolling, oxidation or fermentation, and drying.

Tea leaves contain more than 700 chemicals compounds which are closely related to human health. Among those are amino acids, vitamins (C, E, and K), caffeine and polysaccharides. Besides that, tea leaves contain abundant of the antioxidant compounds. The antioxidant compounds are chemical compounds which inhibit the oxidation process. Polyphenols, flavonoids, and catechins are the examples of antioxidant compounds commonly present in tea. The natural antioxidant has the ability to prevent oxidative stress induced by an imbalance between generation and removal of reactive oxygen species as well as retardation of various chronic diseases in the human body (Ozsoy *et al.*, 2008).

Several studies have been carried out regarding the antioxidant properties of tea and its benefits to human health (Yen and Chen, 1995; Chan *et al.*, 2007). According to Shoji *et al*, (2006), tea polyphenols were found to prevent blood glucose level by inhibition of glucose uptake in the intestine and suppress the increase in blood glucose level after a meal in a diabetic patient. Furthermore, tea possesses various elements which are known to be beneficial for human health. In addition, tea is capable of normalizing blood pressure as well as preventing coronary heart disease, cancer, and several other illnesses (Vinson and Zhang 2005; Hertog *et al.*, 1993).

Recently, utilization of inexpensive residuals of fruits and vegetables has received great attention, since these by-products contain a high amount of phenolic compounds. According to Ignat *et al.,* (2011), reported that some by-products from fruits and vegetables could be potential sources of antioxidants. Interestingly, spent tea is a by-product generated in the beverage production industry which could rapidly accumulate into large amounts of bio-waste, which leads to waste management issues. Spent tea contains various beneficial bioactive compounds such as polyphenolic compounds, organic acid, and edible dietary fibers, which can be recycled and reused.

Hogan *et al.* (2010) stated that waste obtained from grape pomace provide numerous beneficial health effects such as anti-hyperglycemic effects in diabetic mice. Besides, other fruits waste such as blueberry pomace can improve metabolic parameter associated with metabolic syndrome (Khanal *et al.,* 2012), while pear pomace will provide anti-adipogenic effects (Rhyu *et al.,* 2014). Therefore, in tea powder waste (spent tea), the phenolic compounds have not been extracted completely from the tea leaves, due to the short time of brewing, which could be beneficial to human health. The spent tea contains some amount of phenolic and possesses antioxidant capability. Therefore, it could beneficial to the environment and leads to energy and cost reduction, as we can transform the agricultural waste into the functional product.

Tea polyphenols have potential health benefits such as antioxidant, low-density lipoprotein cholesterol lowering effect, and detoxification (Weisburger, 1999). A study conducted by Ramesh *et al.*, (2009), stated that the tea polyphenols probably can reduce the blood glucose level through the inhibition of carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase enzymes. For instance, Hara and Honda and Hara (1993) conducted the inhibition of α-amylase of tea polyphenol in black tea leaves. In another study, Tiwari and Rao (2002) reported the hypoglycemic effect of phytochemicals such as inhibition of carbohydrate hydrolyzing enzyme, manipulation of glucose transporters, β-cell regeneration, and enhancing the insulin-release activity in their studies.

In spent tea leaves, the high content of polyphenols provides an opportunity to be utilized in the food industry. This waste could potentially turn into a healthy ingredient, as the high amount of dietary fibers contained in spent tea leaves could be a beneficial intake in functional food products. By taking into account the combination of the high amount of antioxidant and excellence in inhibition of carbohydrate-hydrolyzing enzymes present in tea and spent tea leaves, this study was conducted to evaluate the potential use of the tea leaves as an ingredient in food industry. The tea and spent tea leaves can be incorporated into bakery products such as biscuits as an ingredient, while there are limited findings on the incorporation of spent tea as a potential diabetic-friendly ingredient in the development of therapeutic food product. After formulating tea and spent tea incorporated biscuits, the glycemic index value was determined in this study.

In general, this study consists of three stages. The first stage is the determination of antioxidant value and chemical composition present in the tea and spent tea leaves. The second stage is to evaluate and compare the hypoglycemic effect of different types of tea leaves (black, oolong, and green tea) and spent tea leaves (spent black, spent oolong and spent green tea) by determining their *in vitro* inhibitory activities. The third stage is the production of biscuit incorporated with tea and spent tea leaves which contain several analyses such as nutritional composition, physical and sensory evaluation. In addition, the hypoglycemic effect of the production of tea biscuit was determined as the glycemic index (GI) values.

1.2 Objective

The main objective was to study the effect of polyphenols present in different types of tea and spent tea leaves on digestion properties *in vitro*, as well as producing a low glycemic index biscuit from tea leaves and spent tea leaves. The specific aims of this study are as follows:

1. To determine the antioxidant activity and mineral elements present in different types of tea leaves and their spent tea leaves, respectively.
2. To study the effect of tea and spent tea leaves on glucose adsorption, glucose diffusion and amylolysis kinetics *in vitro.*
3. To analyze the effect of tea and spent tea leaves on carbohydrate hydrolyzing enzyme (α-amylase and α-glucosidase) *in vitro.*
4. To develop functional biscuit incorporated with selected tea and spent tea leaves, and to determine the nutritional compositions, texture evaluation and sensory acceptability of the biscuit.
5. To determine the glycemic index (GI) value of biscuits incorporated with selected tea and spent tea leaves *in vivo.*

1.3 General overview

PHASE 1: Antioxidant activity and chemical compositions of tea and spent tea leaves.

Sample collection of tea leaves and preparation of spent tea leaves

Black tea (BT)/ Spent black tea (SBT)

Oolong tea (OT)/ Spent oolong tea (SOT)

Green tea (GT)/ Spent green tea (SGT)

↓

Preparation of extract

Water extraction

Ethanol extraction

Analysis

↓

1. Determination of total phenolic content
2. Determination of total flavonoid content
3. Determination of ferric reducing/ antioxidant power (FRAP) assay
4. Determination of free radical scavenging activity and inhibition
5. Determination of phenolic content using High performance liquid chromatography (HPLC)
6. Determination of mineral content by Inductively Coupled Plasma Emission Spectrophotometer (ICP-OES)

PHASE 2: Effect of tea and spent tea leaves on the digestion properties.

Sample collection of tea leaves and preparation of spent tea leaves

Black tea (BT)/ Spent black tea (SBT)

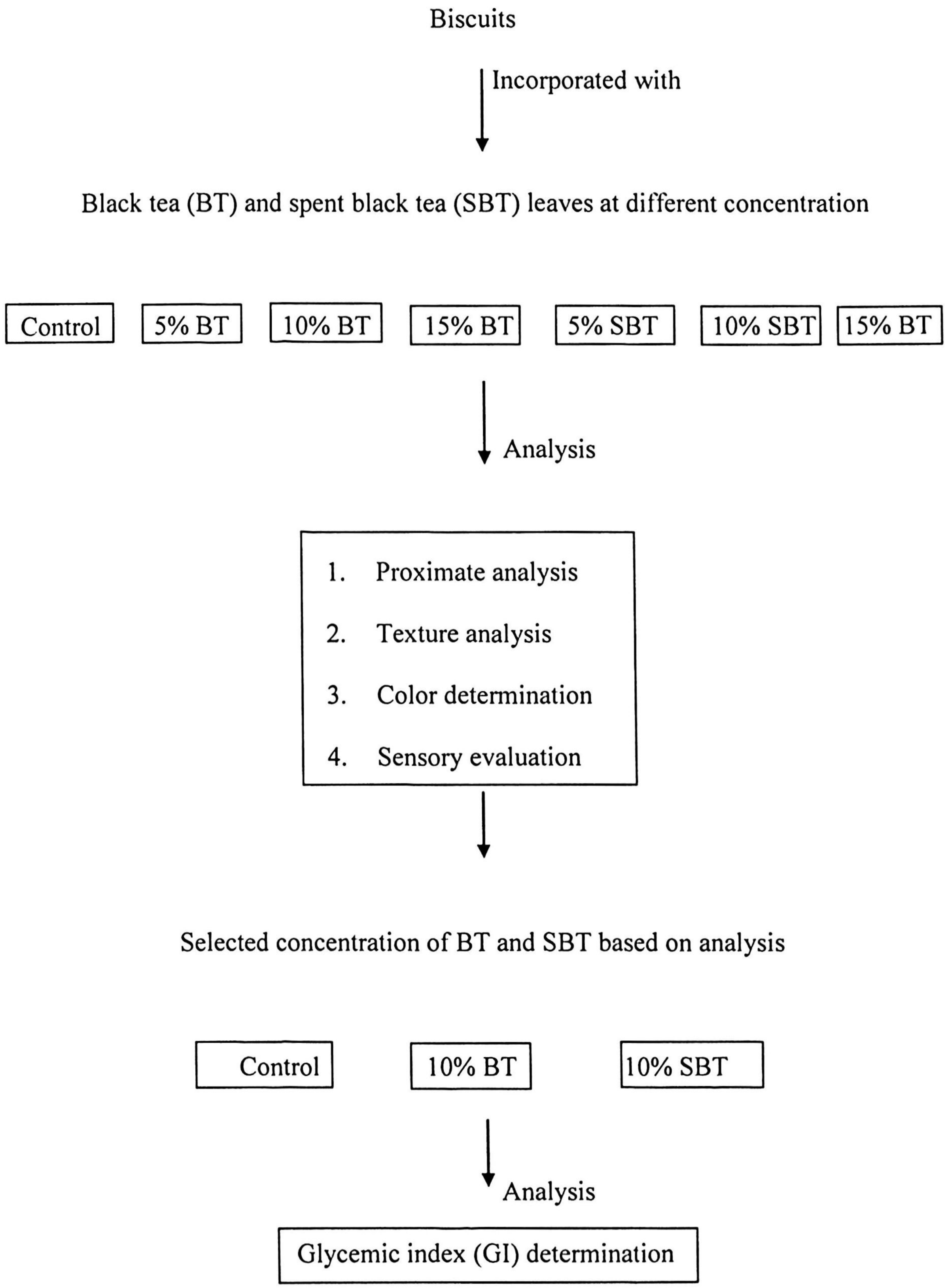
Oolong tea (OT)/ Spent oolong tea (SOT)

Green tea (GT)/ Spent green tea (SGT)

↓ Analysis

1. *In vitro* glucose adsorption capacity
2. *In vitro* glucose diffusion
3. *In vitro* amylolysis kinetics of starch digestion
4. Assay of a-glucosidase inhibition activity

PHASE 3: Glycemic index (GI) determination of biscuits incorporated with black tea and spent black tea leaves.



CHAPTER 2

LITERATURE REVIEW

2.1 History of tea

Tea (*Camellia sinensis*) is a genus of flowering plants in the family *Theaceae*. The tea plant is cultivated on hill slopes with heavy annual rainfall. It has been used in China for more than 5000 years as a medicinal herb to purify the toxins and disease by improving resistance. Approximately 3000 million kg of tea are produced and consumed each year (Khan and Mukhtar, 2007), while over 1000 million kg is produced in China.

Malaysia is a country in Southeast Asia, located in the north of Indonesia on the archipelago between mainland Asia and Australia. Malaysia produces small quantities of tea, while its total production in 2008 was about 0.1 % of the global production, which is placed as the 24th largest producer of tea (FAOSTAT Production Crops, 2010). In Malaysia, the total domestic consumption is around 14000 metric ton, while Boh and Sabah plantations are the main producers of tea products in Malaysia.

The most popular type of tea in the world is black tea. In many cultures, when people request a tea, they are referring to black tea. Outside of Southeast Asia, the majority of the tea produced and consumed is black tea. Black tea is grown in many countries and in various styles and grades. It is difficult to generalize the flavor and aroma of black teas. Black tea is the only type of tea that is widely classified into grades of tea. It is a widespread assumption that black tea is stronger, bitterer and contains higher amount of caffeine in comparison to green teas. The strength of tea depends on how it is brewed and the style and grade of tea.

2.2 Tea manufacturing process

Major amount of teas consumed in the world can be subdivided according to the manufacturing procedures into three major types which are green, oolong and black tea (Chen *et al.*, 2009). The classification of tea is based on a most important characteristic which is the degree of fermentation (Wang *et al.*, 2008). Green tea is an unfermented product containing a relatively large amount of polyphenolic compound compared to the oolong tea and black tea, where oolong tea is partially fermented and black tea is fully fermented (Chaturvedula and Prakash, 2011). Common processing terms are withering, rolling, oxidation or fermentation, and drying.

2.2.1 Withering

Fresh tea leaves are normally harvested by hand plucking or mechanical plucking. The fresh tea leaves usually are selected from the first two leaves and the bud of the growing tea shoot. The newly picked leaves are thinly spread to dry during withering. According to Graham (1992), withering is a process of lowering the moisture content in fresh leaves and breaking down the structural integrity of the leaves to generate the unique tea aroma. This process can take about 10 to 16 hours at a temperature of around 20 to 24 °C and relative humidity of 60 to 70 %, depending on the wetness of the leaf.

For the production of black and oolong tea, the fresh tea leaves undergo withering until their moisture content is reduced to around 55 % of the original leaf weight, whereas for green tea production, the tea leaves only withered in hot air

(Muhktar and Ahmad, 2000). Thus, factors such as the thickness of fresh tea leave spread and the time of withering are dependent on the type of tea being produced (Sharma and Rao, 2009). The main aim of this process is to reduce the water content, thus at the end of this process, the tea leaves should be flexible enough to be rolled.

2.2.2 Rolling

From the withering racks, the leaves are now twisted and rolled so that the leaf cells are shattered while shaking might be used sometimes. The rolling process of tea leaves plays an important part in the final grade of tea (He *et al.*, 2007). Oils are released with this rolling process which provides the tea its distinctive aroma. The juices that are released remain on the leaf. The leaves can be rolled with machinery or by hand. It can be executed mainly by two methods, the Crush, Tear and Curl (CTC) or Orthodox method (Harbowy *et al.*, 1997).

In the CTC method, the crushes of tea leaves are further consistent with the high yielding process, while it produces smaller tea leaves as its sharp teeth machine will cut the leaf then tear it to release the juices that contain the tea flavor. In the Orthodox method, the production of high-quality teas is achieved as tea leaves are prepared by hand rolling or by applying weight or contraction to the leaves. The rolling process is designed to provide a little damage on the leaf structure.

2.2.3 Fermentation

"Fermentation" is mainly defined as the oxidation of catechin in modern tea science. During fermentation, the bioactive polyphenol in tea leaves undergoes enzymatic oxidation resulting in the formation of theaflavins and thearubigins (Lin *et al.*, 2003). In the oxidation, the broken tea leaves are laid out on trays or in a cool and humid environment for up to 2 hours to ferment. During fermentation, the chemical constituents present in tea leaves are allowed to absorb oxygen so that it will undergo enzymatic oxidation reactions.

This process is initiated once the tea leaves membranes were broken during the rolling process. The tea leaves will undergo a natural browning reaction catalysed by enzymes endogenous which will cause the leaves to turn into bright copper in colour (He *et al.,* 2007). Due to the fermentation process, color and aroma of tea leaves change markedly and the degree of fermentation is the main deciding factor whether to produce green, oolong or black tea.

2.2.4 Drying

After fermentation, the tea leaves are dried so that the oxidation process can be stopped. The drying process is performed by passing the broken fermented tea leaves slowly through hot air chambers (He *et al.,* 2007). During the process, all the moisture is evaporated and the tea leaves turn to dark brown or black. At this stage, the aroma of tea leaves will be changed from a pungent plant to the familiar earthy tea perfume (Ito *et al.,* 2002). In black tea manufacturing, drying is important in order to terminate the enzymatic oxidation reaction. The enzymes are inactivated due to the loss of water content. Normally, the tea leaves are first dried at a high temperature, in the range of 100 – 110 °C, and then dried at a lower temperature of around 70 – 75 °C (He *et al.,* 2007).

2.3 Types of tea

There are several types of teas, according to their processing methods. In this study, green tea, oolong tea, and black tea were studied.

2.3.1 Green tea

Green tea is produced by drying and steaming the fresh tea leaves in order to inactivate the polyphenol oxidase (Willson, 1999). Thus, the process of green tea production is the shortest. In the green tea production, withering is initially executed, followed by steaming of the tea leaves for prevention of oxidation. The rolling process to break the cell membrane and oxidation process are skipped in the green tea production.

The last step is drying and steaming of the tea leaves, executed by passing them through a hot cylinder or by passing them through high-pressure hot steam. This process is performed in order to hindering the activity of the enzymes and halting the fermentation process. Normally, the green tea leaves remain green and the steaming process is performed to reduce the water content by about 60 – 70% (Hampton, 1992).

In the production of green tea, there are two steps in the drying process. The first drying process is to reduce the water content to 30-35% and to darken the fluid extracted from the leaves. The second drying process is to improve the forming of the rolled tea leaves (He *et al.*, 2007).

2.3.2 Oolong tea

Oolong tea production is similar to black tea, which undergoes a process known as *wu-long* or *wulong,* most regularly consumed in China and Taiwan (Weisburger, 1999). It is known for its pleasant nutty floral aftertaste and soothing aroma (He *et al.,* 2007). The first two steps are withering and rolling. Instead of rolling, sometimes shaking is done to bruise the outer edges of the leaves. The oxidation period for oolong tea is half of the black tea (Muhktar and Ahmad, 2000).

Once the edges of the tea leaves become reddish brown and the centre remains green, the oxidation process is stopped by firing or a process known as drying. For oolong tea, the tea leaves are heated at a higher temperature so that they can be preserved longer due to the lower water content.

2.3.3 Black tea

Black tea is the most common form of tea in Southern Asia and in many African countries. Westerners refer to it as *black tea,* since the tea leaves used to brew it are usually black in color, while Chinese people call it *red tea* because the actual tea liquid is red.

Both the leaves and brewed black tea tend to be dark in colour, while some black teas have a golden colour. The black tea tends to contain more tannin, a chemical compound that provides the characteristic dark colour of the tea. The tannins are actually a form of antioxidant; catechins of green tea transformed during undergo oxidation (Yamanishi, 1995).

The black tea process undergoes the most stages. Once the leaves are picked, they are left to wither for several hours. After the leaves are rolled, oils from the leaves are brought to the surface. The aromatic oils aid in the oxidation process, which last for several hours (Reeves *et al.,* 1987). The last step consists of placing the leaves in an oven with high temperatures. As soon as the leaves are 80% dry, the leaves complete their drying over wood fires.

The tea leaves are rolled and grounded completely to break their cells so that the process of fermentation can be evenly carried out for 14 to 30 days. This result in the production of teas which are brownish or sometimes black in colour and sorted according to size. The larger grade is considered as "leaf grade" and smaller "broken grade" are usually used for tea bags.

2.4 Major polyphenol compounds of tea

Polyphenol is vegetable tannins that could be found in a wide variety of foods and beverages such as tea, coffee, wine, and some fruits (Charlton *et al.*, 2002). The content of the phenolic compound will influence the astringency and bitterness of the foods and beverage. According to Bravo (1998), polyphenols are products of the secondary metabolism of plants and the presence of polyphenols in plant foods is mainly influenced by genetic factors and environmental conditions such as the degree of ripeness, germination, variety, the degree of fermentation during the manufacturing process, and storage of the plants. The content of polyphenols in the plant varies from a few milligrams to hundreds of milligrams per 100 g of fresh sample weight (Scarlbet and William, 2000).

Polyphenols can be divided into at least 10 different classes such as phenols, benzoquinones, phenolic acids, acetophenones, hydroxycinnamic acids, phenylacetic acids, flavonoids, lignin and xanthones (Harbone, 1989). The main tea phenolic acid is gallic acids, while it contains a certain amount of caffeine as well. The caffeine content varies greatly from different tea types and is highly dependent on the method in which the tea is brewed. Normally, black tea assumes to contain more caffeine than green tea and others types of tea. Green tea is believed to have the greatest number of total phenolic and flavonoid contents, since it will not undergo fermentation or oxidation process (Wheeler and Wheeler, 2004).

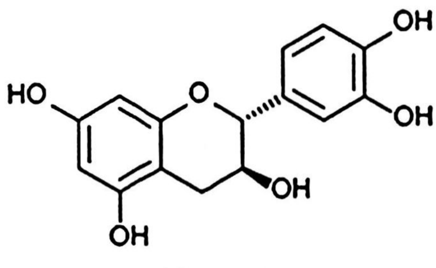
Tea contains caffeine (3-4%), theaflavins (1.5-2.0%), amino acid (theanine, 2%), sugar (4%), and minerals (5%) that are necessary for human health (Jabeen *et* *al.*, 2015). The different amount chemical composition in different types of tea is attributed to several factors including geological location, fertilizers utilization, climatic conditions, and most importantly the processing and packaging of tea leaves (Hussain *et al.*, 2006).

Green tea contains various polyphenols, while the most important flavonoid is catechin. Oolong tea consists of catechins at the level of 8 to 20% of the total dry mass (Graham, 1992). In black tea processing, about 75% of catechins in tea leaves undergo enzymatic oxidation which leads to the formation of theaflavin and thearubigin (Hara *et al.*, 1995).

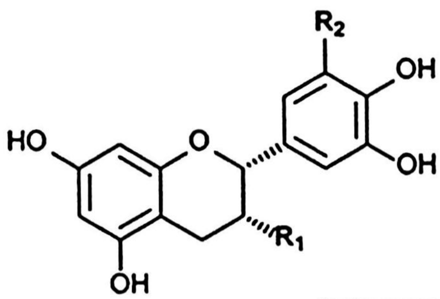
2.4.1 Catechin

Tea extracts are powerful antioxidants while the major tea catechins are (-)-epicatechin, (-)-epigallocatechin, (-)-epigallocatechin gallate and (-)-epicatechin gallateal (Salah *et al.*, 1995) as shown in Figure 2.1. The main dietary sources of catechins are green tea, cocoa beans, and some fruits (Chun *et al.*, 2007). The catechin content is dependent on the geographical location and growing condition of the tea, including soil, climate agriculture, and fertilizers used.

Catechins present in the tea are responsible for several biological activities, while contributing to the taste and astringency of tea extracts (Scharbert et al., 2004). The rich source of catechins especially epigallocatechin gallate (EGCG) is hypothesized to possess multiple benefits for human health (Khan *et al.*, 2006).

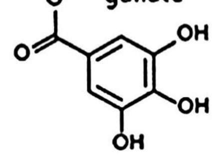


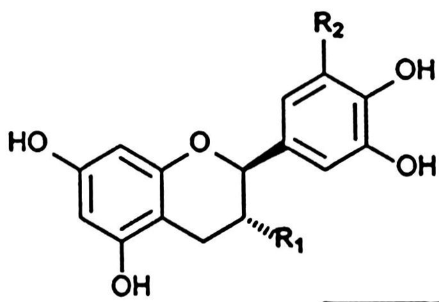
(+)-catechin



	R_1	R_2
(-)-epicatechin	OH	H
(-)-epigallocatechin	OH	OH
(-)-epicatechin gallate	*gallate	H
(-)-epigallocatechin gallate	*gallate	OH

*gallate





	R_1	R_2
(+)-gallocatechin	OH	OH
(+)-catechin gallate	*gallate	H
(+)-gallocatechin gallate	*gallate	OH

*gallate

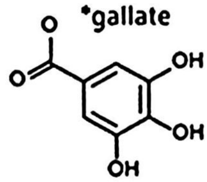


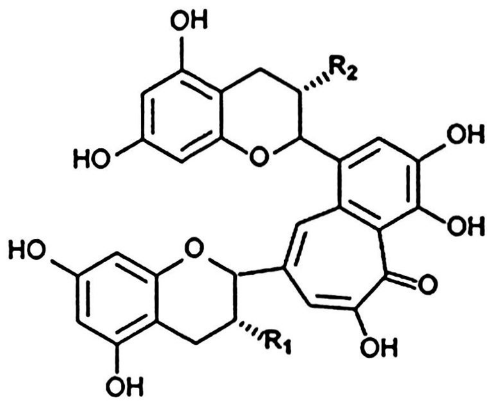
Figure 2.1: Chemical structure of catechins

2.4.2 Theaflavins and Thearubigins

Black tea contains approximately 1 to 4 % of theaflavins and catechins (Lee *et al.*, 2002). The black tea or fermented tea contains a mixture of catechins, theaflavins, and thearubigens produced during the fermentation process of tea leaves. Throughout the fermentation, flavan-3-ols undergo hydrolyzation by polyphenol oxidase enzyme in order to enhance the conversion of the catechins into theaflavins and thearubigins (Wheeler and Wheeler, 2004) as shown in Figure 2.2.

Theaflavins is a soluble oxidation product and orange-red astringent dimer complex which attribute to the colour and taste of the black tea. Thus, theaflavins levels are positively correlated with the quality of black tea (Ullah *et al.,* 1984). According to Luczaj and Skrzydlewska (2004), the main groups of theaflavins are theaflavin and theaflavin-3-gallate.

Thearubigins is less soluble and contributes to the colour and taste of black tea. It is a more complex oligomeric flavonoids compared to theaflavins. According to Sharma and Rao (2009), the thearubigins are responsible for some biological activities and contribute to the reddish colour of the tea extract.



	R_1	R_2
Theaflavin	OH	OH
Theaflavin-3-gallate	•gallate	OH
Theaflavin-3'-gallate	OH	•gallate
Theaflavin-3,3'-digallate	•gallate	•gallate

•gallate

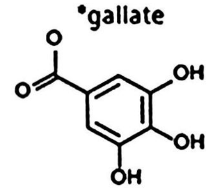


Figure 2.2: Chemical structure of Theaflavins

2.4.3 Flavonols

Flavonoids are widely distributed in plants such as vegetables and fruits. The classification of flavonoids can be divided into six groups which are flavones, flavonol, flavanone, isoflavone, flavan-3-ol, and anthocyanidin groups (Havsteen, 1983).

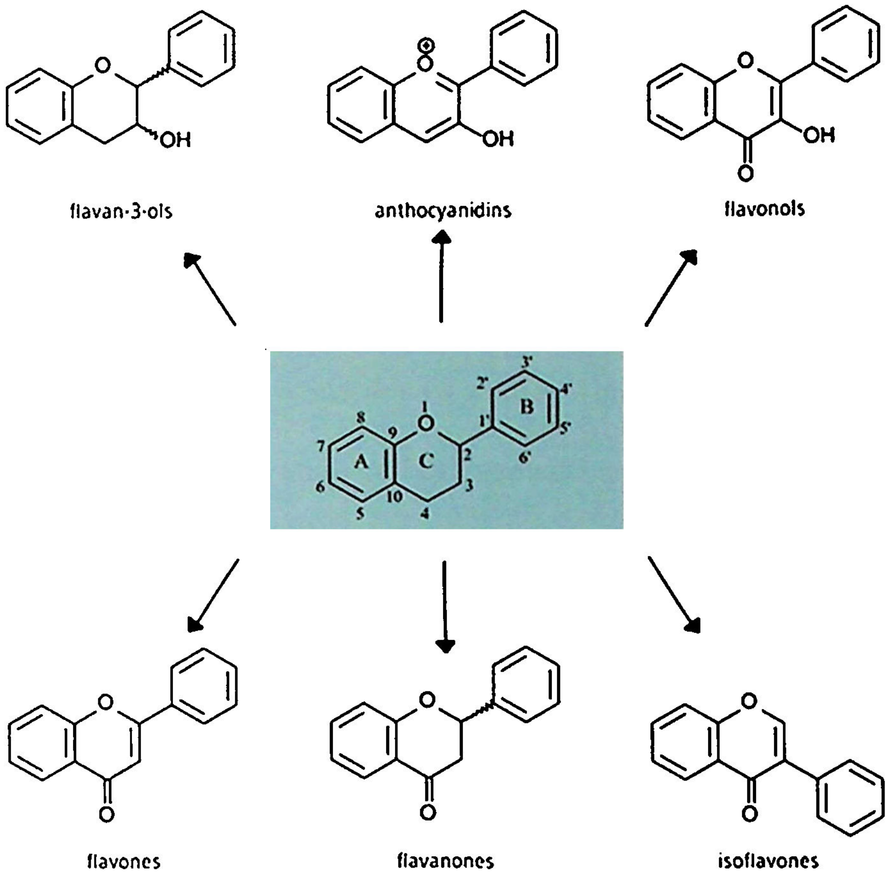


Figure 2.3: Chemical structure of flavonoids

2.4.4 Beneficial biological activities of tea polyphenols

Drinking of a tea beverage is enjoyable, safe, and economically affordable. The traditional Chinese medicine has recommended that consumption of a tea drink may enhance the health problems such as headaches, body aches, digestion, depression, and detoxification, as well as contributing to recovering from some illnesses (Yang and Landau, 2000). A prospective epidemiological study performed in Japan stated that men and women who reported drinking 6 or more cups of green tea per day had one-third less incidence of type 2 diabetes mellitus over 5 years (Iso *et al.*, 2006).

Many studies have been performed and reported regarding the effectiveness of tea on improving the insulin sensitivity (Kwon *et al.*, 2008), maintaining healthy blood pressure (Brownlee, 2005), preventing blood clots (Davies *et al.*, 2003), and reducing the risk of cardiovascular disease (Sharma and Rao, 2009). These studies are experimentation with *in vitro* and *in vivo* studies which lead to the conclusion that tea has protective effects for a variety of health condition.

One of the therapeutic approaches is to prevent absorption of carbohydrate after food intake in order to prevent the diabetes mellitus which characterized by hyperglycemia caused by insufficient or inefficient insulin secretion and associated with hydrolyzing reaction in carbohydrate. According to Kwon *et al.* (2008), a natural antioxidant can inhibit some starch digestion enzymes such as a-amylase, a-glucosidase, thus it can control glucose absorption and reduce the associated hypertension. In addition, tea polyphenols could prevent diabetes by increasing the insulin activity. Moreover, it was claimed that the phenolic antioxidant has the potential to reduce hyperglycemia-induced pathogenesis linked to cellular oxidation stress.

Furthermore, *in vivo* studies verified that treatment of diabetic (induced by STZ rats) with 1.25% of black tea for 3 months decreased the formation of diabetic cataracts (Thiagarajan *et al.*, 2001). The intake of tea in STZ rats prevents the activation of sugar aldose reductive pathway and thus may be effective in retarding diabetes and incidence of diabetic cataracts. Anderson and Polansky (2002) reported that active compound of EGCG in green tea increases insulin activity and stated that addition of lemon to tea did not affect the insulin potential activity, while addition of 50 g of milk per cup decreased the insulin potential activity, similar to 90%. They also stated that the theanine present in black tea can help to control blood pressure and reduce the stress.

Obesity has increased at a rapid rate in recent years. Current interest in the role of functional foods in weight control has focused on plant ingredients capable of interfering in weight management control (Dullo *et al.,* 1999). The effects of long-term consumption of tea catechins have been widely studied, while some researchers suggest the potential of green tea in body weight control. In addition, caffeine and theanine have been found to strengthen polyphenol effects on body weight control and fat accumulation in mice (Zheng *et al.*, 2004)). *In vitro* studies with green tea extracts reported significant inhabitation of the gastric lipase and interfere in fat emulsification process, which occurs before enzymes action for lipid intestinal absorption (Juhel *et al.,* 2000). Green tea also exhibits a fatty acid which possesses inhibition activity (Sang *et al.,* 2004). However, Wu *et al.,* (2003) indicated that an inverse relationship may exist among regular green tea consumption, body fat percentage, and body fat distribution, especially for subjects who have maintained the habit of tea consumption for more than 10 years.

2.4.5 Effect of polyphenol on carbohydrate metabolism

Digestion is a process involving the hydrolysis of large and complex organic molecules of food into smaller and preferably water-soluble molecules which can be easily absorbed by the gastrointestinal tract for utilization by the organism. Digestion takes places in several organs such as the mouth, duodenum, and small intestine. Principal dietary carbohydrates are polysaccharides which consist of starch (plant) and glycogen (animal), disaccharide (lactose and sucrose) and monosaccharide (glucose and fructose). In the digestion process, the complex polysaccharides of carbohydrate will convert into monosaccharide by hydrolyzing enzymes such as a-amylase, a-glucosidase, and sucrose.

In mouth, digestion of carbohydrate takes place when saliva contains salivary a-amylase hydrolyzes the a-1,4-glycosidic bonds. Thus, it produces maltose, glucose, disaccharide, and maltotriose. The salivary a-amylase enzyme requires chloride ions for activation and need an optimum pH of around 6.6 to 6.8. In the duodenum, pancreatic juice that consists of pancreatic a-amylase has a similar action to salivary a-amylase enzymes. It will produce disaccharide (maltose and isomaltose) and oligosaccharide during this stage. In the small intestine, the intestinal juices that contain a-amylase will catalyze lactose, isomaltose, maltose, and sucrose into simple forms such as glucose, galactose, maltose, and fructose. Then, the absorption of simple carbohydrate will occur mostly in the duodenum and upper jejunum of the small intestine, while only monosaccharides are absorbed by the intestine.

In the absorption process, insulin is responsible for the uptake of glucose in peripheral tissues including muscle, adipose tissue, and kidney, by promoting the conversion of glucose into glycogen in the liver and inhibiting the lipolysis in adipose tissue. Hanhineva *et al.,* (2010) stated that the rise of glucose concentration in blood promotes secretion of insulin from the β-cells of the islets Langerhans in the pancreas. Insulin will stimulate the glucose uptake in skeletal muscle and in adipose tissue, whereas in the liver insulin trigger conversion of glucose to glycogen as storage so that the hepatic glucose output rate will be reduced.

Dietary polyphenols may influence glucose metabolism by stimulating peripheral glucose uptake in insulin sensitive and non-insulin sensitive tissue. Many studies showed plant extracts rich in polyphenols could enhance glucose uptake (Hanhineva *et al.,* 2010) and *in vivo* animal studies reported that major polyphenol isolated from green tea enhance the glucose tolerance in diabetic mice, reduce hepatic glucose production, and improve pancreatic function (Wolfram *et al.*, 2006).

2.5 Tea spent and applications

Spent tea is the main bio-waste by-product that is generated in the beverage production industry that could rapidly accumulate into large amounts, leading to waste management issues. Spent tea contains various beneficial bioactive compounds such as polyphenolic compounds, organic acid, and edible fibers, which could be recycled and reused. Several studies reported that waste obtained from natural sources has many beneficial health effects such as grape pomace which has anti-hyperglycemic effects in diabetic mice (Hogan *et al.*, 2010), blueberry pomace which could improve metabolic parameters associated with metabolic syndrome (Khanal *et al.*, 2012), and pear pomace which has anti-adipogenic effects (Rhyu *et al.*, 2014).

Generally, the optimal steeping of tea varies widely depending on the type of tea and the time preferred by the consumer. Brewing the tea is a complex art as well as a matter of personal taste. In general, tea leaves tend to taste best when brewed with boiling water, while some are brewed with water slightly or below the boiling point. The time of brewing varies depending on the type of the tea, consumer preference and the taste of final tea beverage. Occasionally, one minute of steeping could be sufficient, whereas some teas may taste best if steeped as long as 5 to 8 minutes.

2.6 Effect of dietary fiber addition in food products

Nowadays, there are numerous by-products from vegetable and fruits incorporated in flour-based products such as bakery products, pasta, and noodles (Anis Jauharah *et al.*, 2014). Vegetable and fruits are the best sources of dietary fibers. Thus, incorporation of vegetable and fruits in bakery product could provide positive or negative effects on nutritional compositions and sensory acceptability as well as the texture profile. Several studies have been perfumed regarding the positive impacts of incorporation of amaranth leaves powder in the dietary fiber of biscuit (Sing *et al.*, 2011) and addition of pumpkin and carrot pomace powder in cookies.

2.6.1 Dietary fiber

According to FAO/WHO (1998), dietary fiber is defined as the polysaccharides of the plant cell wall such as cellulose, hemicelluloses, pectins, and hydrocolloids. Starch consists of digestible starch polysaccharides like amylose and amylopectin, and indigestible non-starch polysaccharides such as dietary fiber. The non-starch polysaccharides can be further subdivided into soluble and insoluble based on chemical, physical, and functional properties (Webb *et al.*, 2008). The soluble fibers consist of pectins, gums, inulin-type fructans, and some hemicelluloses are dissolved in water forming viscous gels that bypass the digestion of small intestine (Lattimer and Haub, 2010). It is easily fermented by the microflora of the large intestine. Furthermore, insoluble fibers are not water-soluble in the human gastrointestinal tract, since they do not form gels attributed to their insolubility and lower fermentation. The insoluble fiber consists of lignin, cellulose, and some hemicellulose (Wong and Jenkins, 2007).