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Oolong Tea: A Critical Review of Processing Methods, Chemical Composition, Health Effects and Risk

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ABSTRACT

Oolong tea (OT) is a traditional Chinese tea (*Camellia sinensis*) and is especially popular in south China. This review is to comprehensively summarize the miscellaneous research that has been done towards to the processing, phytochemistry, health benefit, and risk of OT. These literatures were carried out not only from different electronic databases but also from text books written in English, Japanese and Chinese, including those traditional records tracing back to the

Tang Dynasty (A.D. 618-907). The full process OT producing is depicted below in this review.

The phytochemistry of oolong tea has been comprehensively investigated. More than 100 chemical compositions have been isolated and identified. In health benefit, OT performs outstandingly in reducing obesity and controlling diabetes explained by modern pharmacological studies. (-)-Epigallocatechin-3-gallate (**6**) in OT prevention of cancerous cells developing. OT can also improve and reduce on heart and vascular disease, protect teeth and bone, function as anti-oxidative and antibacterial agents. This review also mentioned the risk, summarized briefly on various forms of toxicity and harmful associated with OT. In short, this review can provided a natural product library of OT, gave inspirations for further new garden systems, designed idea on quality, bioactivity-oriented screening. In addition, it is suggested more scientists and education is necessary to guarantee the stability and safety of drinking OT.

Key Words

Oolong tea; Processing methods; Chemical composition; Health effects; Risk

1. Introduction

“Coffee or tea?” is a familiar question, asked in restaurants and at homes around the world. In Asia, the beverage of choice has been tea for centuries. Nowadays, tea can be classified into different types, such as green, yellow, white, oolong, black and Pu-erh. All are made from the leaves of the same species, *Camellia sinensis*. The significant differences of tea types derive from growing conditions of the plant, harvesting procedures, and processing of the leaves (Yi *et al.*, 2015). Of all the types, oolong is unique because researchers showed it can reduce obesity and control diabetes (Mo *et al.*, 2007; Wang *et al.*, 2010; Hayashino *et al.*, 2011). Oolong tea is semi-oxidized tea. Varying degrees of oxidation made oolong tea varieties are endless possibilities. This review focuses on oolong tea. It comprehensively summarized all aspects of the tea from where and how it is grown, and processed to the research that has been done on its health benefits.

The name oolong tea came into English language from its Chinese name (烏龍) meaning “black dragon tea.” In some ancient books, oolong tea used the term “blue tea.” There is a widely accepted explanation of the origin of the Chinese name. The name originally had nothing to do with dragons, rather it was named after Wu Long who discovered oolong tea by accident. Wu

Long was a tea grower and hunter. One day, he was distracted by a deer after collecting a good load of tea. He stopped tea picking and tried to slay the beast. By the time he remembered to return to the tea, it had already started to oxidize. He did not want to let tea go to waste, therefore he finished tea process. Surprisingly, this tea was mellow and aromatic, unlike any tea he had tasted before. Before long Wu Long's tea was known throughout the province and started using a homophone name (oolong tea) for Wu Long's tea (Li and Bian, 2013).

Oolong tea is a traditional type of tea in China, very popular throughout Asia. It can be found in restaurants, vending machines, corner shops and supermarkets, and is served both hot and cold (Pettigrew, 2007). Oolong tea is semi-oxidized tea, giving a taste between black and green tea (Fanaro *et al.*, 2012). Oolong tea leaves are showing green in the center and red at the edge, and it is commonly called green leaves with red edging (Chen, 2011). There is a wide variety of oolong tea, and its origin at Fujian province of China (Zhang and Du, 2015). In the past few years, oolong tea is known to provide robust health benefits if drunk frequently (Lin and Chen, 2012). With these benefits, the purpose of drinking OT changes from quenching thirst to gaining health benefits. In fact, oolong tea is a complex chemical mixture and different chemicals will give different health benefits. The purpose of this article is to review and

interpret recent research relatively on sources of oolong tea processing, chemical composition, as well as the benefits of oolong tea consumption.

1.1 Distribution

The first oolong tea appeared between the Ming (A.D. 1386 - A.D. 1644) and Qing (A.D. 1644 - A.D. 1912) Dynasty. Fujian province of China is the place of its origin. However, it is still unknown whether it comes from north or south Fujian (Ye, 2009). Oolong tea is now produced in four different places in China: north and south Fujian Province, Guangdong Province, and Taiwan (Huang *et al.*, 2008; Wu, 2011). It is also produced in some other Southeast Asia countries, such as India, Nepali, Thailand, Vietnam and Indonesia (Lin *et al.*, 1998; Pripdeevech and Machan, 2011; Darjeeling district government, 2012; Chen and Lin, 2014; Mishra *et al.*, 2014; Theppakorn *et al.*, 2014). Regions of production in Asia are shown in **Figure 1**. Oolong tea is also being cultivated in central African countries (Kuo *et al.*, 2005), as well as on the North Island of New Zealand (Chen, 2011; Fraser *et al.*, 2014).

1.2 Growing condition

Every plant has its unique habitat. Oolong tea tree is no exception. Suitable climate, terrain and soil not only promote crop yield, but also the quality of tea leaves.

Oolong tea prefers warm climate with a temperature range of approximately 10-30°C. It does not adapt well to excessively hot or cold places. Generally, this plant grows best in foggy, drizzly seasons when soil is moist. Places with 1,000 to 1,250mm annual rainfall are ideal for growing oolong tea (Chen and Lin, 2014).

There is a Chinese saying which goes, “Good tea comes from the highlands”, so tea tree is always planted in elevations of 300 to 2,130 metres. However, some varieties of oolong tea are best planted in elevations of 200 to 300 metres. Da Hong Pao sometimes is called “rock teas” because of the distinctive terroir of the mountainsides where they are grown; **Figure 2** is showing this growing condition. This oolong tea grown in the rocky, mineral-rich soil is highly prized. Some other oolong tea requires deep and fertile soil. It grows best in red yellow soil with pH4.5-6.5 (Huang and Yang, 2005).

1.3 Processing

Firstly, to produce good oolong tea, the tea leaves must be collected at a particular time. Secondly, the leaves must be processed with proper methods. Processing is the most important factor in determining the quality of oolong tea. The processes in oolong tea can be generally classified into seven steps, sunning and withering, fermenting, panning, rolling, firing,

final-firing and packing. The full process is depicted below in **Figure 3** (Roberts, 1985; Varnam and Sutherland, 1994; Xue, 2003; Huang and Yang, 2005; Huang *et al.*, 2008; Ye, 2009; Mukhtar and Ahmad, 2000; Chen, 2011; Chen and Lin, 2014; Zhu, 2015).

The quality of a cup of oolong tea is determined by a number of factors. In addition to the variables of production, the way of using the tea leaves to make tea is important. The different ways of brewing oolong tea are shown in **Table 1** (Gong, 2008; Ye, 2009; Wu, 2011; Wang, 2013; Shi, 2015).

In 21st century, researchers suggested that hot and cold water steeping of oolong teas did not affect its antioxidant activity (Venditti *et al.*, 2010). Among these new technologies, oolong tea becomes more hygienic, and easy to bring, brew, drink, clean, storage and transport for those people who worried about diabetes and obesity.

1.4 Varieties

This review will introduce six of the most common varieties of oolong tea in China (**Table 2**). These are Tie Guan Yin (TGY), Da Hong Pao (DHP), Phoenix Dan Cong Tea (PDC), Dongfang Meiren (DM), Pou Chong Oolong (PCO) and Dong Ding (DD). The degree of oxidation (Huang *et al.*, 2008; Wu, 2011) and oxidation process (**Figure 5**) (Cheng, 2008; Lin,

2009) both directly affect the characteristics of the final product. The degree of oxidation can be up to 90%, depending on the variety and production style (Xue, 2003).

1.5 Conservation

With the rapid economic development and gradually improving living standards, people drink oolong tea is not just to quench thirst but also to improve health. Wild population of the plants used to make oolong tea is dwindling rapidly. The Chinese government has already taken steps to protect oolong tea. It has been prohibited to pick leaves from six mother trees of DHP since 2008. Asexual reproduction is now being used to propagate DHP plants in tea plantations (Cai, 2015). To meet increasing demand, researchers are attempting to increase the yield of oolong tea by reducing damage from diseases and insects, improving fertilizers, and developing systems for rapid propagation and zero tillage.

Diseases and insects are the major problems of tea production, as both yield and tea quality drop when tea trees suffering from disease or insect damage. Excessive humidity and poor drainage are the factors that render tea plants susceptible to disease. Therefore, researchers are seeking ways to improve ventilation and increase sunlight in tea gardens. To limit the spread of disease, growers should cut off any infected stems at the first sign of disease. If the disease

becomes more serious, tea garden should not use organic cultivation (Huang and Yang, 2005).

Insect damage is another issue. Scientists and growers have solved this problem with biological and physical controls. Biological control agents use for oolong tea include *Trichogramma ostrinae* against *Homona magnanima*; *Amblyseius womersleyi* against *Tetranychus kanzawai*, and *Mallada basalis* against small bugs. In terms of physical control, interposed stickers can be used to control leafhopper, spiny whitefly, thrips and aphids. Some growers also use lemongrass, tobacco or chili powder as pest control products (Wu, 2011). Grower education and more scientific reports are needed for bio-control. These researches can show growers that natural predators can help to reduce or eliminate pests. Eliminating the use of pesticides can also solve the problem of pesticide residues in oolong tea.

Modified fertilization can also increase the yield of oolong tea. A report from 1991 shows that when nitrogenous (N), phosphate (P), potash fertilizers (K) and rape cake (RC) were applied at proper amounts and ratios, the oolong tea leaves will have higher amounts of amino acids, polyphenols, catechins and total sugars (Lin *et al.*, 1991). Another mathematical research model for the fertilizing suggests that the best ratios for fertilizing tea plants are $N:P:K:RC = 3:1:3:2$ or $N:P:K:RC = 2:1:2:3$. The effect of the ratios was different according to the age of the plants, and researchers showed that K is the main factor for stem growth in young oolong tea trees. K

and RC also affect the quality (Zhang and Liang, 2004). Nowadays, organic farming that is without pesticides or chemical fertilizers is common. Therefore, researchers started testing organic fertilizer on oolong tea. The result showed that organic fertilizer (rabbit excrement, 4 treatments) 10kg with special microorganisms 20g could improve the output of oolong tea leaves (Tu *et al.*, 2006).

Establishment of new tea plantations and improvement of old tea plantations can help to meet the growing demand for oolong tea. The total tea planting area in Fujian increased from 109 thousand hm^2 in 1980 to 200 thousand hm^2 in 2011. Improved designs of old tea plantations included the tri-dimensional ecological tea garden and a compounded biological cycle tea garden. Using scientific fertilization and zero tillage can also increase the production (Yang *et al.*, 2012).

1.6 Quality control

In the process of oolong tea differentiation, distinguish oolong tea from its perspectives of color, aroma and taste. It is extremely perceiving quality. According to the research using 31 oolong tea samples, the concentration of chlorophyll correlated strongly with the appearance of the infused leaf. Besides, the concentration of total free amino acids and theanine positively

correlated with perceived taste. In the view of its chemistry, glutamic acid (**95**) and catechins are the major type of chemical constituents in most oolong teas. Researchers have found that the principal components, including glutamic acid (**95**), (-)-epicatechin (**5**) and total catechins, could be used to characterize the quality of oolong tea. However, interpretation of the results remains open to debate (Wang *et al.*, 2010). Further work could be to determine more scientific indicators for judging quality, like using a colorimeter to characterize tea color. In addition, quality assessment equipment, such as electronic nose, electronic tongue and lipid membrane taste sensors, could be designed to assess the quality of tea.

2. Chemical composition

Oolong tea contains several different chemical compounds that can improve human health and treat illness. A diversity of these compounds has been identified, including alkaloids, polyphenols, flavonoids, aromas, vitamins, minerals, amino acids, protein, polysaccharide and organic acids.

2.1 Alkaloids

Theobromine (**1**) and caffeine (**2**) have been found in oolong tea (Hosoda *et al.*, 2003; Yi *et al.*, 2015). One study also found theophylline (**3**) (Chen *et al.*, 2003). Their structures are illustrated in **Figure 6**.

2.2 Polyphenols

Many studies have reported finding a variety of polyphenols in oolong tea. These different flavonoids can be categorized into four sub-groups, namely catechins, tannins, flavonols and flavonol glycosides.

The catechins in oolong tea include epigallocatechine (**4**), (-)-epicatechine (**5**), (-)-epigallocatechin gallate (**6**), (-)-epicatechin gallate (**7**), (-)-gallocatechine (**8**), (+)-catechine (**9**), (-)-gallocatechin gallate (**10**), (-)-catechin gallate (**11**) (Hosoda *et al.*, 2003; Theppakorn *et al.*, 2014; Yi *et al.*, 2015). (-)-Epigallocatechin 3-*O*-(3-*O*-methyl) gallate (**12**) has also been reported (Kurata and Takao, 2012; Fei *et al.*, 2014; Zhang and Weng, 2014). The corresponding structures are depicted in **Figure 7**.

Poanthocyanidina type tannins (**Figure 8**), hydrolyzable tannins (**Figure 9**), tea pigments (**Figure 10**) and other tannins (**Figure 11**). Among them, the compounds are: procyanidin B-2

(13), procyanidin B-2 3,3'-di-*O*-gallate (14), epigallocatechin-(4 β →8)-epicatechin 3-*O*-gallate (15), epigallocatechin 3-*O*-gallate-(4 β →8)-epicatechin 3-*O*-gallate (16), epigallocatechin-(4 β →8)-epigallocatechin 3-*O*-gallate[prodelphinidin B-2 3'-*O*-gallate] (17), epigallocatechin 3-*O*-gallate-(4 β →8)-epigallocatechin 3-*O*-gallate[prodelphinidin B-2,3'-*O*-gallate] (18), epicatechin-(4 β →8)-epigallocatechin 3-*O*-gallate (19), epicatechin 3-*O*-gallate-(4 β →8)-epigallocatechin 3-*O*-gallate (20), procyanidin B-3 (21), procyanidin B-4 (22), catechin-(4 α →8)-epigallocatechin (23), galocatechin-(4 α →8)-epicatechin (24), galocatechin-(4 α →8)-epigallocatechin[procyanidin B-4] (25), catechin-(4 α →8)-epigallocatechin 3-*O*-gallate (26), prodelphinidin B-4 3'-*O*-gallate (27), procyanidin B-5 3,3'-di-*O*-gallate (28), epigallocatechin 3-*O*-gallate-(4 β →6)-epigallocatechin 3-*O*-gallate[prodelphinidin B-5 3,3'-di-*O*-gallate] (29), epicatechin 3-*O*-gallate-(4 β →6)-epigallocatechin 3-*O*-gallate (30), epigallocatechin 3-*O*-gallate-(4 β →6)-epicatechin 3-*O*-gallate (31), epiafzelechin 3-*O*-gallate-(4 β →6)-epigallocatechin 3-*O*-gallate (32), theogallin (33), β -glucogallin (34), strictinin (35), epitheafagallin 3-*O*-gallate (36), theaflavin (37), theaflavin 3-*O*-gallate (38), theaflavin 3'-*O*-gallate (39), theaflavin 3,3'-di-*O*-gallate (40), theasinensin A (41), theasinensin B (42), theasinensin C (43), theasinensin F (44), theasinensin D (45), 8-C-ascorbyl

(-)-epigallocatechin 3-*O*-gallate (**46**), oolonghomobisflavans A (**47**) and B (**48**), prodelphinidin B-2 3'-*O*-gallate (**49**) and oolongtheanin 3'-*O*-gallate (**50**) (Hashimoto *et al.*, 1989; Mukhtar and Ahmad, 2000; Tanaka *et al.*, 2010; Lunagariya *et al.*, 2014; Caballero *et al.*, 2015).

Three flavonols were observed in aged oolong tea leaves, myricetin (**51**), quercetin (**52**) and kaempferol (**53**) (Lee *et al.*, 2008). 19 flavonol glycosides compounds have been found in oolong tea, including myricetin rhamnoglucoside (**54**), myricetin 3-*O*-rutinoside (**55**), myricetin 3-*O*-galactoside (**56**), myricetin 3-*O*-glucoside (**57**), quercetin glucorhamnogluconide (**58**), quercetin 3-*O*-rhamnoglucoside (**59**), quercetin dirhamnogluconide (**60**), quercetin rhamnogalactoside (**61**), quercetin 3-*O*-rutinoside (**62**), quercetin 3-*O*-galactoside (**63**), quercetin 3-*O*-glucoside (**64**), kaempferol glucorhamnogluconide (**65**), kaempferol 3-*O*-rhamnoglucoside (**66**), kaempferol 3-*O*-galactoside (**67**), kaempferol dirhamnogluconide (**68**), kaempferol 3-*O*-rutinoside (**69**), kaempferol 3-*O*-glucoside (**70**), teaghrins-1 (**71**) and teaghrin-2 (**72**). In addition, prechafuroside A (**73**) and B (**74**) were discovered in oolong tea (Ishida *et al.*, 2009; Lo *et al.*, 2014; Jiang *et al.*, 2015). Both flavonol and flavonol glycoside structures are shown in **Figure 12**.

2.3 *Tea polysaccharides*

Oolong tea polysaccharide (OTPS) is a kind of water-soluble polysaccharide isolated from oolong tea. Monosaccharides of OTPS were composed of D-rhamnose, L-arabinose, D-galactose, and D-glucose (Chen *et al.*, 2009a; Jiang and Xiao, 2015). **Table 3** summarizes the molecular weight (MW), monosaccharide composition, and protein content of OTPS.

2.4 *Minerals*

Herrado and Gonzalea (2001) used inductively coupled plasma atomic emission spectroscopy (ICP-AES) to analyse oolong tea samples, and have found aluminum (Al), barium (Ba), calcium (Ca), copper (Cu), iron (Fe), kalium (K), magnesium (Mg), manganese (Mn), sodium (Na), strontium (Sr), titanium (Ti) and zinc (Zn) in oolong tea.

Shen and Chen (2008) tested oolong tea with an inductively coupled plasma mass spectrometer (ICP-MS), and they discovered heavy metals in oolong tea, including chromium (Cr), cadmium (Cd), and lead (Pb). Semi-metals such as arsenic (As) were also found. Six essential trace elements cobalt (Co), Cu, Fe, Mg, Zn and selenium (Se) have been found in oolong tea.

Zhong *et al.* (2016) tested 25 Chinese tea samples including oolong tea with high-resolution continuum source graphite furnace atomic absorption spectrometry, and determined nickel (Ni) in oolong tea.

2.5 Others

Most of the floral aspects of the aroma of oolong tea are derived from β -primeverosides. One was identified by Guo in the earlier 1990s, namely geranyl 6-*O*- β -xylopyranosyl- β -D-glucopyranoside (**75**). Later, (S)-linalyl (**76**), 2-phenylethyl (**77**), benzyl disaccharide glycosides (**78**), trans- (**79**) and cis-linalool 3,6-oxide 6-*O*- β -D-xylopyranosyl- β -D-glucopyranosides (**80**) were identified as aroma precursors from oolong tea (Guo *et al.*, 1994; Moon *et al.*, 1994; Ogawa *et al.*, 1997). In addition, nerolidol (**81**), α -farnesene (**82**), linalool (**83**), β -ionone (**84**), jasmine lactone (**85**), indole (**86**), and some oxidized forms, namely dihydroactinidiolide (**87**), geranylacetone (**88**), hexanoic acid-3-hexene ester (**89**), geraniol (**90**), γ -decalactone (**91**), and methyl jasmonate (**92**), were identified by gas chromatography-mass spectrometry (GC-MS) (Tokitomo *et al.*, 1984; Kinugasa and Takeo, 1990; Lv *et al.*, 2014). The structures are shown in **Figure 13**.

Apart from the components mentioned above, other constituents include amino acids (**Table 4**) like L-theanine (**93**), the highest content amino acid in oolong tea, is showing in **Figure 14** (Ekborg-Ott *et al.*, 1997; Horanni and Engelhardt, 2013; Turkozua and Sanliera, 2015); organic acids such as gallic acid (**94**), glutamic acid (**95**), quinic acid (**96**), 5-galloylquinic acid (**97**), 5-caffeoylquinic acid (**98**), 3-*p*-coumaroylquinic acid (**99**), 4-*p*-coumaroylquinic acid (**100**), 5-*p*-coumaroylquinic acid (**101**) and strictinin (**102**) (Dou *et al.*, 2007). Non-metals such as fluorine (F) (Chen, 2011), proteins and complex sugars including oligosaccharides or polysaccharides (Chen 2011; Lin and Chen, 2012; Stanway, 2013) have also been found in oolong tea. Several vitamins including vitamin U, hydrophilic vitamin B, C as well as hydrophobic vitamin A, E and K have been identified in tea leaves (Ohtsuki *et al.*, 1984; Gou, 2005; Rahman *et al.*, 2013).

3. Health benefits

Historically, tea has been used as medicine in China. According to the records in “A Supplement to Materia Medica”, tea was a good medicine that could cure hundreds of diseases (Liang, 2013). Although tea is not a miraculous cure as the records suggest, many scientific analyses shows that most of the chemical ingredients in tea are related to many effective

medicines. The distinctions between oolong tea and other tea are shown at **Figure 15** (Kuo *et al.*, 2005; Karori *et al.*, 2007; Kong *et al.*, 2014; Wang *et al.*, 2016). The most distinctions are chemicals contain differently. The relationship between chemicals and health benefits are described below by different modern medicine researches.

3.1 Reduces obesity and controls diabetes

Early reports suggest that oolong tea contains caffeine that can enhance lipolysis via acting on lipid droplets but not on hormone-sensitive lipase (HSL). This indicates that oolong tea may be an effective crude drug for the treatment of fatty liver and obesity caused by a high-fat diet (Han *et al.*, 1999). Another research reports that polymerized polyphenols (OTPP) from oolong tea extract inhibit pancreatic lipase (Nakai *et al.*, 2005). One study using animals reports that oolong tea extracts may play a role in weight loss; the effect is dose-related and does not involve change in food eaten. Also, it was found that with the increasing doses, the increase of rat body weight gradually slowed. Therefore, the medium dosage appears to be most effective (Mo *et al.*, 2007). Meanwhile, research was done on the anti-obesity effects of oolong tea for diet-induced overweight and obese subjects. This study found that the extract and catechins of oolong tea could reduce body weight and decrease body fat content via lipid metabolism. Besides, oolong tea consumption (drinking) may prevent obesity (He *et al.*, 2009).

Some researchers also found that the polysaccharides contained in oolong tea had good hypoglycemic effect (Zhang *et al.*, 2008). Late on in the research of Wang *et al* (2010), results showed that oolong tea polysaccharides had significant effects on lower the blood sugar by blinding the α -amylase enzyme (up to 26.18%). There have been other studies, both animal experiments and clinical trials, assessing the use of oolong tea to reduce the risk of diabetes and to control diabetes. The details are shown in **Table 5**.

3.2 Prevents development of cancerous cells

This section will highlight some studies that document the anti-cancer effects of catechins (**4-12**) from oolong tea and oolong tea extracts in different models, including *in vitro* and *in vivo* pre-clinical models, animal models (**Table 6**), and human case studies (**Table 7**).

GABA stands for gamma-aminobutyric acid, an amino acid occurs naturally in *Camellia sinensis*. It is believed that increased GABA can decrease stress and anxiety (Iversen, 2004; Anju et al., 2014). Therefore, GABA in oolong tea can act as a natural sleep aid. Nowadays, a new technique for processing oolong tea is available in Japan and Taiwan and the product namely oolong GABA tea, which contains high amount of GABA (Lee and Peng, 2013). However, by taking GABA by oral, the GABA level will not increase in the brain. The reason

is GABA cannot pass the blood-brain barrier and get into the central nervous system (Boonstra *et al.*, 2015). Due to this reason, researchers have changed the research direction of GABA. Al-Wadei *et al* (2013) used MTT assays, cell migration assays, Western blotting and immunoassays in the research and identified GABA can act as a promising agent for the prevention of pancreatic ductal adenocarcinoma (PDAC). Researchers also suggested that GABA might have preventive effects on these non-PDAC cancers.

3.3 Improves cardiovascular health and reduces heart disease

A study in Taiwan has been carried out on the effect of tea drinking over several decades on the risk of newly diagnosed hypertension in 1507 subjects (711 men and 796 women). Study subjects were 20 years or older who did not have a hypertensive history. This study found that the habitual consumption of moderate strength oolong tea (i.e., drinking 120mL/day of oolong tea for more than one year) can reduce the risk of hypertension. Therefore, the researcher recommended that extractions of oolong tea can reduce the risk of developing hypertension significantly in the Chinese population (Yang *et al.*, 2004).

Results of a study done in the late 1990s showed that (-)-epicatechin gallate (**7**) and (-)-epigallocatechin gallate (**9**) in tea extracts might account for their hypocholesterolemic

effect (Yang and Koo, 1997). In addition, Zhu *et al.* (2002) also showed that certain fractions of oolong tea could inhibit erythrocyte hemolysis. Meanwhile, some researchers have suggested that oolong tea-polymerized polyphenols (OTPP) had the effect of suppressing postprandial serum triglyceride increases. This indicates that OTTP-enriched oolong tea (beverage) has remarkable suppressive effects on the postprandial serum triglyceride and hyperlipidemia elevations, and oolong tea (beverage) is supported as being useful for risk reduction of life-style related diseases (Yuji *et al.*, 2004; Toyoda-one *et al.*, 2007).

In addition, another study found that long-term oolong tea consumption (drinking) was associated with the reduced risk of ischemic stroke in southern Chinese population. At the same time, this study indicated that the risk of ischemic stroke was significantly lower among subjects who regularly drink one or two cups of oolong tea daily (Liang *et al.*, 2009).

Other research found that long-term drinking oolong tea with its average polyphenol content of gallic acid (**94**), caffeine (**2**), epigallocatechin (**4**), epicatechin (**5**), epigallocatechin gallate (**6**), galocatechin gallate (**10**), epicatechin gallate (**7**) and catechin gallate (**11**), can significantly increase plasma adiponectin levels and low-density lipoprotein (LDL) particle size, as well as decrease plasma levels of total cholesterol and hemoglobin Alc significantly. Thus, this

research supports the belief that long-term intake of oolong tea may have beneficial effects on the progression of atherosclerosis in patients with coronary artery disease (CAD) (Shimada *et al.*, 2004). Besides, another study on a population in Shantou found that long-term oolong tea consumption (drinking) can reduce the risk of hypercholesterolaemia (H-TC) and hypertriacylglycerolaemia (H-TAG), which indicates that long-term oolong tea consumption (drinking) may be associated with the reduction risk of dyslipidaemia (Yi *et al.*, 2014).

3.4 Functions as an antioxidant

Research indicates that different tea types have different *in vitro* antioxidant functional power, and this power strongly depends on the total phenolics content in the tea (Benzie and Szeto, 1999; Hou *et al.*, 2006). Moreover, epigallocatechine (**4**) and (-)-epigallocatechin gallate (**6**) account for about 70% of oolong tea total catechins. This explains why oolong tea can show effective antioxidant function (Yang *et al.*, 1998). In this regard, more research has been done on green and black tea than on oolong tea. However, according to the results from the study of Zhu *et al.* (2002), an oolong tea water extract (OTE) and its individual fractions presented 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities and reducing power, and can provide protection against DNA oxidation.

The *in vitro* antioxidant test showed that oolong tea polysaccharide (OTPS) had certain scavenging activity on hydroxyl radical, superoxide anion radical and DPPH radical. The IC_{50} were 0.1289 g/L, 0.9181 g/L and 0.5233 g/L. The scavenging rate has positive correlation with OTPS concentration. Although the antioxidant activity of OTPS are lower than vitamin C (IC_{50} = 0.0205 g/L, 0.2287 g/L and 0.0255 g/L), oolong tea is still a good antioxidant drinks (Zhou et al., 2011).

3.5 Oral health

Research shows that oolong tea can protect teeth. Early research has suggested that the oolong tea extract (OTE) containing polymerized polyphenols can significantly inhibit plaque deposition in humans (35 volunteers, aged 18-29). Furthermore, these findings support the conclusion that the OTE preparation could be useful for controlling not only dental plaque formation but also subsequent dental caries development in humans (Ooshima et al., 1994).

According to Ooshima et al's (1998) animal experimental report, the crude preparation (OTE) of oolong tea polyphenols (including the polyphenol fractions OTF1 and OTF6 of OTE) have cariostatic activity even after the establishment of *Streptococcus sobrinus* in the oral cavity and is more effective in preventing caries than drinking water. In addition, there are some evidence

that OTE contains other cariostatic substances, besides polyphenols, that reduce the virulence of *Streptococcus sobrinus* (Hamilton-Miller, 2001).

Besides, researchers like Sasaki *et al.* (2004) and Yoo *et al.* (2011), have reported that oolong tea extract has antibacterial effect on oral streptococci, including *Streptococcus mutans* and *Streptococcus sobrinus*. Their results showed particularly significant antibacterial activity against *Streptococcus mutans*, and suggested that the antibacterial activity of oolong tea extract is caused by a synergistic effect of entirely monomeric compounds include EGCG (**6**) and GCG (**10**), which can easily bind to proteins.

3.6 Others

Oolong tea has other health benefits such as: 1) effect on males, 2) effect on bone, 3) effect on the brain, 4) effect on muscle.

A report showed that drinking oolong tea is an effective treatment for erectile dysfunction due to hyperlipidemia (Chen *et al.*, 2011).

Besides, oolong tea has the function of protecting bone mineral density. One study shows oolong tea has also been shown to protect bone mineral density in postmenopausal Han Chinese women (Wang *et al.*, 2014).

Studies show that oolong tea has higher level of L-theanine (**93**) when compared to green tea and black tea (Ekborg-Ott *et al.*, 1997; Turkozua and Sanliera, 2015). The L-theanine (**93**) is found in oolong tea as amino acid, and it can block glutamate receptors from accepting L-glutamic acid in the brain. Cortical neuron excitation will not occur while this amino acid binds to those receptors. In this way, the brain can keep the mind at rest in order to decrease stress (Kimura *et al.*, 2007; Yoto *et al.*, 2012). Therefore, oolong tea has anti-stress effects due to L-theanine (**93**). Moreover, L-theanine (**93**), EGCG (**6**) and theaflavins (**37-40**) may protect the brain from Alzheimer's disease through multiple mechanisms including the actions on upstream events such as amyloid formation (Ehrnhoefer *et al.*, 2008; Bieschke *et al.*, 2010) and downstream events such as oxidative stress and inflammation (Song *et al.*, 2012).

As mentioned above (**Table 4**), leucine contents up to 1.22 mg/g in oolong tea. Leucine was one major amino acid for muscle building. Muscle protein synthesis is through the mTOR pathway, Leucine is an activator for this pathway. Researchers suggested that leucine can work in both neonates (Columbus *et al.*, 2015) and the elderly (Barkoukis, 2016). The experiment was look at muscle protein synthesis when additional leucine is added to the diet or to a test meal. Results showed that leucine was able to reliably increase muscle protein synthesis. The

next studies should be tried the other two amino acids with three branched chain and namely isoleucine and valine for increase muscle protein synthesis.

4. Risks

Oolong tea is regarded as a kind of health tonic; however, drinking too much or drinking at an unsuitable time can instead harm health. In this part, we will explain potential risk of oolong tea.

4.1 Overdose

Drinking a large volume of oolong tea may dilute gastric juices, increase the work load of the digestive system, slow down digestion, and subsequently may cause gastritis, indigestion, bloating, abdominal pain, and possibly duodenal ulcer (Li, 2015).

Fluoride found in oolong tea is considered as an essential micro-nutrient for human health to prevent dental caries and promote healthy bone growth, but excess fluoride could lead to detrimental health problems in human, especially fluorosis of the teeth and skeletal fluorosis (Chan *et al.*, 2013). A case study reported in 2013 showed a hard-to-diagnose case of severe bone damage in a 47-year-old woman due to drinking large volume tea, from 100 to 150 tea

bags daily (estimated fluoride intake, >20 mg per day), over almost 20 years. Also because of brittleness, all her teeth had been extracted. Researchers found out the fluoride level in her blood were astronomically high, suggested this patient have skeletal fluorosis and recommended skeletal fluorosis lead to teeth lose and brittleness (Kakumanu and Rao, 2013).

According to a study published in 2002, results of analyzing oolong tea using high pressure liquid chromatography showed that oolong tea has oxalate contents ranging from 0.23 to 1.15 mg/g. In the same study, researchers suggested that oxalate in tea has the potential to bind to a significant proportion of calcium in the milk (Charrier *et al.*, 2002). Oxalate is a known chemical to cause kidney stones in idiopathic stone-forming patients or even kidney failure when ingested in excessive amounts (Knauf *et al.*, 2013; Nagaraju *et al.*, 2013; Kullin *et al.*, 2016). An unusual case of kidney failure was reported in April 2015. A 56-year-old Arkansas man presented to the hospital in May 2014 with weakness, fatigue and body aches. Doctors determined his kidneys were badly clogged and inflamed, but he had no family or personal history of kidney disease. After being questioned, this patient admitted to drinking a large volume of tea daily. Researchers concluded that the kidney damage was almost certainly due to excessive consumption of oxalate in tea (Syed *et al.*, 2015).

4.2 Adverse reactions

Drinking tea at extreme temperature, either hot or cold, can damage health. Drinking tea which is too hot will irritate and inflame the throat, esophagus and stomach lining. Always drink high temperature oolong tea can cause permanent scarring and rawness to these areas. A study in 2009 published in a British medical journal showed that drinking very hot tea (higher than 70°C) increased the likelihood of oesophageal cancer (Islami *et al.*, 2009; Islami *et al.*, 2009). On the other hand, drinking icy oolong is also not good for health. Many studies have established that cold beverages slow gastric emptying in mammals. El Ouazzani and Mei (1979) found out that cold solutions (10-12°C) infuse into duodenum of cat more slowly than warm solutions (46-49°C). Likewise, a research showed that warm liquids (45°C) emptied from the canine stomach faster than cold liquids (5°C) (Teeter and Bass, 1982). In 1988, a report carried out with six healthy men who had no past history of gastrointestinal disease showed the onset of digestive absorption for cold drinks (4°C) was slower than for the control temperature drinks (37°C). Researchers suggested that when cold drinks get into stomach, a mammal's body is forced to use energy in order to warm up that liquid inside the stomach to match its natural temperature. This action will rob the energy needed to properly ingest (Sun *et al.*, 1988; Sun *et al.*, 1995). In some cases, human may experience bradycardia and syncope after drinking cold

liquids (Brick *et al.*, 1978; Armstrong *et al.*, 1985; Casella *et al.*, 2009; Popkin *et al.*, 2010; Kim *et al.*, 2012).

A cohort study conducted on a Japanese male population has shown different outcomes after long term consumption and short-term consumption of oolong tea. The report found that long term consumption is in accord with the onset of diabetes whereas short term consumption shows anti-hyperglycemic effect in diabetic patients (Hosoda *et al.*, 2003; Hayashino *et al.*, 2011). The oolonghomobisflavans A (**47**) and B (**48**) isolated from the leaves of oolong tea showed inhibitory activities against pancreatic lipase (Nakai *et al.*, 2005). Another study has shown a causal link between the over-consumption of oolong tea and the development of hypokalemia, atrioventricular block, QT prolongation and incessant non-sustained ventricular tachycardia (Toshiya *et al.*, 1999).

A case control study was conducted in Kaohsiung, Taiwan between August 1996 and June 1997. This research showed that oolong tea consumption was associated with increased bladder cancer risk. The odds ratio of bladder cancer for oolong tea drinkers was 3.00 (95%, confidence interval 1.20 to 7.47). The result also showed the longer tea drinking history the greater risk (Lu *et al.*, 1999). Another research study from the University of Glasgow on 6,016 men who were

heavy tea drinkers found that men who drank seven or more cups of tea a day had a 50% higher risk of prostate cancer. This cohort study was done between 1970 and 1973, and included followed-ups of up to 37 years (Shafique *et al.*, 2012).

4.3 Food or drug interactions

Drinking tea and eating food at the same time is very common, such as afternoon tea in England or “Yum Cha” in Hong Kong and Guangzhou province of China. Sometimes oolong tea leaves are cooked with food. Recent research shows that tea may interact with some food. For example, Wu *et al.* (2009) found out that it implies a health risk with drinking oolong tea while having with ginseng as medicinal. The experiment involved green tea, oolong tea and black tea; the results showed that the total ginseng saponins were significantly low in all groups who drank tea. However, drinking oolong tea with ginseng is a perfect good idea if you are eating the ginseng for flavor and not its medicinal properties.

Large amounts of oolong tea should not be drunk before a meal, nor immediately after a meal. It is not suitable to drink oolong tea immediately after a meal because research shows that tea drinking limits the absorption of non-haem iron. Researchers suggested that healthy people with no risk of iron deficiency do not need to restrict their tea drinking, but anaemia patients and

people at risk of iron deficiency must pay attention to drink tea between meals and to wait at least one hour after eating before drinking tea (Nelson and Poulter, 2004).

As mentioned above, drinking tea while having meals is common in the world nowadays. Therefore, further research should focus on the interactions between food and oolong tea, or the whole tea family. Researchers should try to discover what kinds of food may interact with oolong tea. Building up an interaction database for oolong tea would be a small but significant meaningful work.

4.4 Special risk groups

4.4.1 Children

In some case, children are not interested in drinking oolong tea due to its astringency. The astringency of oolong tea mostly results from polyphenol-protein interactions, mainly catechins (Chen *et al.*, 2014). Catechins are also known to non-specifically bind food and other biological proteins. Ferruzzi *et al.* (2012) has shown that flavan-3-ol type compounds (**4-12**) interact with physiological proteins in food. Furthermore, the results of Wu *et al.* (2013) showed that the binding interaction between EGCG (**6**) and bovine β -lactoglobulin (milk protein) is mainly hydrophobic, and the complex is stabilized by hydrogen bond. Moreover, one study has

reported that small tea polyphenols will have more effect on milk α -casein protein than larger ones (Bourassa *et al.*, 2013). According to the polyphenol-protein interactions, child drinking oolong tea might experience inadequate protein intake resulting in malnutrition. As mentioned above, oolong tea may affect iron absorption, and iron is a necessary part of children's diet because it is needed for proteins that carry oxygen in the blood. In addition, low levels of iron can lead to fatigue and low immunity (Nelson and Poulter, 2004).

4.4.2 Older adults

Warfarin is a common anticoagulant in elderly patients; it is normally used in the prevention of thrombosis and thromboembolism. Its chemical structure is very similar to vitamin K. As vitamin K can be found in oolong tea, it could possibly interfere with the operation of the blood pigment, and reduce the risk of blood clots in patients (Goodchild and Donaldson, 2009).

A research study in 2013 has suggested that transient ischemic attack-like symptoms in older adults could possibly be attributable to one or more components of oolong tea; the onset of symptoms was very quick after the consumption of oolong tea in this study. Analysis of the oolong tea showed no evidence of any extraneous chemicals that could lead to the symptoms.

The researchers had recommended strong oolong tea may lead to acute transient ischemic attack-like effects and concluded that more research is needed (Layher-Jr *et al.*, 2013).

4.5 Caffeine

Caffeine contents (in mg) of all tea types are shown in **Table 6** (Stanway, 2013). Caffeine (**2**) can cause sleep disorders, and it might cause headache, anxiety, agitation, chest pain, and ringing in the ears when drunk for a long time and/or in fairly high doses. Tannins in oolong tea will combine with caffeine (**2**) after steeping, and will subsequently be released in the human body more slowly than caffeine from coffee. Therefore, its effects last longer than coffee's (Stanway, 2013). Drinking oolong tea on an empty stomach or drinking concentrated oolong tea will increase the absorption of caffeine (**2**) by the intestines and stomach. Excessive caffeine (**2**) will lead to hyperfunction of the adrenal cortex and influence the vitamin B1 absorption in human body (Yang, 2014). Caffeine (**2**) may speed up the heartbeat in order to increase blood pressure. Additionally, intake of too much caffeine (**2**) can easily cause headaches, irritability, and stomach problems (Hara *et al.*, 2014). It can cause the human cerebral cortex to overreact, leading to cerebral vasoconstriction and insufficiency, and ultimately, possibly, headaches (Li, 2014). Moreover, oolong tea may increase the amount of calcium (Ca) that is flushed out in the

urine due to its high caffeine (2) content. Too much caffeine (2) can lead to osteoporosis by interfering with the absorption of Ca (Heaney, 2002).

In particular, caffeine (2), which is a bit higher in oolong tea than in other types of tea except black (**Table 8**), may cause problems.

A case control study took place in Sapporo, Japan, from 2003 to 2004. This study found out that the caffeine (2) intake during early pregnancy was related with enhanced risk of recurrent pregnancy loss only among susceptible women (Sata *et al.*, 2005). Caffeine (2) is possibly safe in pregnant women when used in daily amounts of less than 300 mg (Higdon and Frei, 2006). This is about the amount in 4-6 cup (8 fl.oz /240ml) of oolong tea according to **Table 8**. Consuming larger amounts of caffeine (2) during breast-feeding is possibly unsafe because caffeine (2) can pass into breast milk. One research study showed caffeine (2) is detectable in breast milk within 15 minutes of consumption, and peaked at about one hour after consumption (Berlin *et al.*, 1984). Therefore, nursing mothers should closely monitor caffeine intake to make sure it is on the low side. High intake of caffeine by nursing mothers can cause sleep disturbances, irritability, and increased bowel activity in breast-fed infants (Higdon and Frei, 2006).

5. Bioavailability and metabolites

Possible beneficial effects of oolong tea are determined by its bioavailability. Bioaccessibility means released and solubility of bioactive compounds during digestion for further uptake and absorption; it is one important factor for bioavailability of oolong tea. The catechins (**4-12**) and theasinensins (**41-45**) both are bioactive compounds in oolong tea. According to Qiu *et al* (2012), the possible routes for absorption of catechins mainly by paracellular pathway and partially by monocarboxylic acid transporter, while theasinensins transport through intestine membrane via tight junction. These catechins and theasinensins are believed to occur in small intestine and metabolized can generate two forms, metabolites and native forms. **Figure 16** shows the outline of a possible metabolism route of oolong tea and **Figure 17** summarizes the *in vitro* and *in vivo* biotransformation of catechins (Scalbert and Williamson, 2000; Kohri *et al.*, 2001a; Kohri *et al.*, 2001b; Meng *et al.*, 2002; Lee *et al.*, 2006; Parada and Aguilera, 2007; Takagaki and Nanjo, 2009; van't Slot and Humpf, 2009; Roowi *et al.*, 2010; Schantz *et al.*, 2010; Takagaki and Nanjo, 2013; Ozdal *et al.*, 2016).

Future efforts should consider the absorbed pathway of theasinensins conjugates. Moreover, the metabolism of catechins and theasinensins in colon are required. In addition, concentration of

theasinensins in oolong tea is higher than other tea types. Therefore should take more research on metabolites *in vivo*, *in vitro* and *in silico* model. Likewise, should come up with more research on the pharmacokinetics for theasinensins type compounds and these 23 compounds which have been reported in **Figure 17**.

6. Conclusion and prospects

This review has documented the distribution, processing, phytochemistry, as well as these health benefits and risks of oolong tea. This review has showed that: **1)** The availability of the various components in oolong tea varies according to how the tea leaves are grown, processed and prepared as tea. **2)** Six major types of oolong tea can be produced from the fresh leaves of *Camellia sinensis*, and oolong teas are different in terms of biochemical quality and sensory attributes. **3)** The most widespread health benefits of drinking oolong tea could be reducing obesity and controlling diabetes. **4)** Oolong tea is considered to be safe and effective against various cancers. However, **5)** adverse effects following the consumption of large amounts or concentrated preparations of oolong tea and caffeine have been reported.

Although increasing interest has prompted more studies on oolong tea, it is still several points are noteworthy. The results reported here point the way for future research. Firstly, in the

studies cited above, most of samples were from Asia. Future research should consider including subjects of different races in order to make samples more representative. Secondly, as EGCG (6) and TF (37) showed effect on cancers, the next projects should look into the absorption in the human organism of EGCG (6) and TF (37). In particular, an *in vitro* Caco-2 cell monolayer model should be established to simulate the intestinal absorption of EGCG (6) and TF (37), in order to study the concentration and time for absorption.

Thirdly, being one of the most popular beverages worldwide, oolong teas should be assessed with regard to its role in the primary and secondary prevention of chronic diseases. Future research needs to outline the actual extent of health benefits, determine the range of oolong tea consumption associated with these benefits, and clarify probable mechanisms of action.

Fourth, development of methods that are more specific and sensitive with more models of representation together with the development of good predictive biomarkers will enable a better understanding of the interaction of oolong tea with endogenous systems and other exogenous factors. Most of the reports above show that catechins are the main chemicals responsible for oolong tea's power to reduce obesity and control diabetes. However, there has been no work to determine which catechin is the most potent. Such research could use ultra-filtration coupled

with high-performance liquid chromatography and quadrupole-time-of-flight mass spectrometry for screening diabetes enzymes such as α -glucosidase inhibitors or α -amylase inhibitors of different catechins from oolong tea (Tao *et al.*, 2015). By using this method, the most potent chemical can be found.

Last but not least, oolong tea is a pleasant, popular, socially accepted, economical and safe beverage. This, together with its health benefits, gave it great potential for becoming an important development of functional food, nutrition and food additives.

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Table 1. The ways of brewing oolong tea.

Time	Ways of brewing	Temperature°C	Method	Tool and equipment
Tang Dynasties	Tea cooking	100	Add oolong tea leaves to boiling water and cook until boiling again	Pot and tea cups
(A.D. 618-907)				
Ming and Qing Dynasties	Tea brewing	95-99	Pour boiling water onto oolong tea leaves directly	Teapot and tea cups
(A.D. 1368-1644)				
Qing Dynasties	Kung-Fu tea	90-95	1)Warm teapot or tea-brewing vessel	Used up to 16 tools and equipment, a basic set showed on Figure 4
(A.D. 1644-1912)			2)rinse the oolong tea leaves with boiling water.	
			3)Add water to make the first brew.	
			4)Add more water for additional brews.	
			5)Aroma appreciation.	
			6)Serving	
1950s till now	Ready-to-drink	Both hot and cold	Open the can or box and drink	None

Table 2. General information regarding the six most common varieties of oolong tea produced in China.

Varieties	Translated English name or common name	Distribution	Degree of oxidation		Comments	
			Taiwan government recommended	Tea grower recommended		References
Pou Chong Oolong (PCG)	Pou Chong Oolong	Taipei in Taiwan	8-12%	Less than 15%	1) A lightly oxidated, usually not baked;	Cheng, 2008;
					2) With slight green tea flavors;	Gong, 2009
					3) With floral and melon fragrances and rich, mild taste.	
Dong Ding (DD)	Frozen Summit	Nantou in Taiwan	15-30%	20-35%	1) Ball rolling technique seals in the sweet floral notes and flavors;	Cheng, 2008;

	Ice Peak				2) Sweet taste accompanied by floral notes; tea is golden and amber color after brew	Gong, 2009
Tie Guan Yin (TGY)	Iron Buddha	Anxi in south Fujian	15-30%	20-50%	1) One of the most widespread oolong tea;	Gong, 2008;
	Rolled Oolong				2) Can be collected 4 times a year, the best is from spring tea period;	Lin, 2009
					3) The fragrance is lasting after brewed and taste sweet.	
Dongfang Meiren (DM)	Oriental Beauty	Hsinchu in Taiwan	50-60%	60-75%	1) A heavily fermented oolong tea;	Xue, 2003;
	White Tip Oolong				2) Always pick tea leaves which	Chen and Lin, 2014

					<p>attacked by <i>Jacobiasca formosana</i>;</p> <p>3) Bright-reddish orange tea liquor produces sweet taste and has natural fruity aromas.</p>	
Phoenix Dan Cong Tea (PDC)	Single Bush	Phoenix Mountain in Guangdong	ND	70-80%	<p>1) Extremely high prices in the top grades;</p> <p>2) The tea color is clear bright yellow and has unique natural fragrance.</p>	Huang <i>et al.</i> , 2008
Da Hong Pao (DHP)	Big Red Robe	Wuyi in north Fujian	ND	85-90%	1) A nearly fully-oxidized oolong;	Gong, 2008

	Wuyi Rock Tea				2) Always grown among the rocks;	
					3) According to the leaf edge become red color after processing, look like wearing a big red robe;	
					4) After brewing, it is very fragrant, with the aroma of orchid.	

ND = no data

Table 3. The molecular weight, monosaccharide composition, and protein in oolong tea polysaccharides.

		Monosaccharide composition (mol% or mole ratios)						
Name	MW/kD	Rha	Fuc	Ara	Gal	Glc	Protein%	References
tOTPS	5.30-100.90	16.20	/	43.70	18.00	21.90	32.70	Chen <i>et al.</i> , 2009a
OTPS2-1	88.77	1.02	1.76	7.05	7.58	2.14	1.10	Ni <i>et al.</i> , 2004
pOTPS	128.00	1.37	/	1.89	1.00	1.30	19.59	Chen <i>et al.</i> , 2009b

tOTPS = total oolong tea polysaccharides, pOTPS = purity oolong tea polysaccharides, Rha =

rhamnose, Fuc = fucose, Ara = arabinose, Gal = galactose, Glc = glucose.

Table 4. Content of amino acid in oolong tea samples.

Amino acid	Oolong tea (n=4) mg/g
Alanine	0.19-0.76
Arginine	0.21-0.69
Asparagine	0.11-1.33
Aspartic acid	0.25-1.37
GABA	0.09-0.97
Glutamic acid	0.92-1.49
Glutamine	0.26-0.76
Glycine	0.06-0.08
Histidine	0.14-0.31
Isoleucine	0.15-0.37
Leucine	0.12-1.22
L-theanine	3.97-14.57
Lysine	0.11-0.43
Phenylalanine	0.10-0.71
Proline	0.08-0.51
Threonine	0.15-0.34
Tryptophan	0.09-0.48

Serine	0.31-0.81
Valine	0.14-0.72

Table 5. Prospective cohort studies of the effect of oolong tea consumption on diabetes

mellitus.

Model(s) [cases/control]	Type of test	Test period	Chemical [cases/control]	Biological results	References
Taiwan men and women [10/10]	randomized crossover design	30 days + 30 days	Cases = OTE 1500 ml/day	Markedly lowered concentrations of plasma glucose	Hosoda <i>et al.</i> , 2003
			Control = Water 1500 ml/day		
Japan men [GroupA = 1036, GroupB = 677/ Control = 3262]	cohort research	5 years	GroupA = OTE 250 ml/day	Long-term consumption of oolong tea may be a predictive factor for new onset diabetes	Hayashino <i>et al.</i> , 2011
			GroupB = OTE 500 ml/day		
			Control = OTE 0 ml/day		
Rat Hepatoma H4IIE Cells	Western blotting (<i>in vitro</i>)	4 hours	OTE and EGCG	Oolong tea contains a fraction with insulin-like activity	Yasui <i>et al.</i> , 2011
Non-obese diabetic Goto-Kakizaki rat	(<i>in vivo</i>)	3 weeks	EGCG 200 mg/kg/day	Increasing diponectin production	Shimada <i>et al.</i> , 2007

EGCG = (-)-epigallocatechin-3-gallate; OTE = oolong tea extract

Table 6. Biological effects of oolong tea in *in vitro* and *in vivo* pre-clinical models, animal models of cancer.

Type of cancer	Chemical compound(s)	Model(s)	Biological end point	Effective dose	Reference
Hepatocellular Cancer	EGCG (6)	Human hepatoma derived PLC/PFR/5 cells (<i>in vitro</i>)	↓cell growth, ↓α-fetoprotein	1-100 μM; 48 h	Nishida <i>et al.</i> , 1994
	EGCG (6)	HepG2 cells (<i>in vitro</i>)	↓cell growth, ↑ODC activity	5--400 μM; 24 h	Ramirez-Mares <i>et al.</i> , 2004
	TF (37), TFGA (38), FGB (39) and TFDG (40)	BEL-7402 human liver cancer cells (<i>in vitro</i>)	↓ cancer cell growth	0.0039, 0.016, 0.063 and 0.25 mg/ml; 72 h	Tu <i>et al.</i> , 2004
	ECG (7) and EGCG (6)	BEL-7404/DOX resistant human HCC cells (<i>in vitro</i>)	↓proliferation, ↓HIF1-α, ↓P-gp	150--4300 μM; 48 h	Liang <i>et al.</i> , 2010
	EGC (4), EC (5), EGCG (6), ECG (7) and OTE	DENA-treated male F344 rats (<i>in vivo</i>)	↓GST-P ⁺ foci	0.05%, 0.1% w/w;	Matsumoto <i>et al.</i> , 1996
				2,6,12 weeks	
	EGCG (6)	HLE tumors in nude mice (<i>in vivo</i>)	↓xenograft tumors, ↑apoptosis, ↓Bcl-2α, ↓Bcl-xL	0.8, 2.5, 7.5 mg/ml;	Nishikawa <i>et al.</i> , 2006
				25 days	
	EGCG (6)	HuH7 xenografts in nude mice (<i>in vivo</i>)	↓Akt, ↓Bcl-xL, ↓ERK, ↓VEGFR-2	0.01%, 0.1%; 5 weeks	Shirakami <i>et al.</i> , 2009

	ECG (7) and EGCG (6)	BEL-7404/DOX xenograft in nude BALB/c mice (<i>in vivo</i>)	↑GST, ↓P-gp, ↑Topo-II, ↓MDR1, ↓HIF-1α, ↑anti-tumor activity of doxorubicin	40, 80 mg/kg; 33 days	Liang <i>et al.</i> , 2010
	EGCG (6)	Male obese and diabetic C57BL/KsJ-db/db mice (<i>in vivo</i>)	↓p-IGF-1R, ↓p-ERK, ↓p-Akt, ↓p-GSK-3β, ↓p-STAT3, ↓p-JNK, ↓TNF-α, ↓IL-6, ↓IL-1β, ↓IL-18	0.1% w/v; 34 weeks	Shimizu <i>et al.</i> , 2011
Melanoma	OTE	B16 mouse melanoma cell (<i>in vitro</i>)	↓ cancer cell growth	1-100 μM; 24 h	Aoki <i>et al.</i> , 2007
Esophageal Cancer	EGCG (6)	KYSE 150 cells (<i>in vitro</i>)	↓protein levels of EGFR and HER-2/neu	5, 10, 20, 50 μmol/L; 24 h	Hou <i>et al.</i> , 2005
Lung Cancer	EGCG (6)	cisplatin-induced lung tumors in A/J mice (<i>in vivo</i>)	Inhibit cisplatin-induced weight loss and lung tumorigenesis	1 mg/ml in tap water;	Mimoto <i>et al.</i> , 1999
				30 weeks	
Stomach Cancer	OTE	Human stomach cancer KATO III cells (<i>in vitro</i>)	Apoptosis by fragmentation of DNA to oligonucleosomal sized fragments	Dose-dependent	Hibasami <i>et al.</i> , 2000
Anti-cancer	OTE	Salmonella/microsome reverse mutation assay (<i>Salmonella typhimurium</i> TA98 and TA100) (<i>in vitro</i>)	Anti-mutagenicity	0.35 mg/plate; 48 h	Yen and Chen, 1996

	OTE, EGCG (6), Gallic acid and Caffeine (2)	Salmonella/microsome reverse mutation assay (<i>Salmonella typhimurium</i> TA100, TA98 and TA97) (<i>in vitro</i>)	Anti-mutagenic activities	Dose-dependent	Hour <i>et al.</i> , 1999
	Oolong tea methanol extract	Salmonella/microsome reverse mutation assay (<i>Salmonella typhimurium</i> TA1535/pSK 1002) (<i>in vitro</i>)	Anti-genotoxic abilities (suppressive effects against tumor gene expression)	Dose-dependent	Ohe <i>et al.</i> , 2001
	EGC (4), ECG (5), EGCG (6), ECG (7), TF (37) and OTE	Male Donryu rats (<i>in vivo</i>)	Inducing apoptosis, and cell cycle arrest	200 μ M; 12,24,48 h	Zhang <i>et al.</i> , 2000

EC = epicatechin; ECG = epicatechin gallate; EGC = (-)-epigallocatechin; EGCG =

(-)-epigallocatechin-3-gallate; EGFR = epidermal growth factor receptor; ERK = extracellular signal

regulated kinase; ESCC = esophageal squamous cell carcinoma; GST = glutathione S-transferase; GST-P

= glutathione S-transferase placental form; HER-2/neu = human epidermal growth factor receptor 2;

HIF1- α = hypoxia inducible factor 1- α ; HNC = head and neck cancer; IL-6 = interleukin-6; IL-1 β =

interleukin-1 β ; IL-18 = interleukin-18; MDR1 = multi drug resistance 1 gene; ODC = ornithine

decarboxylase; OTE = oolong tea extract; p-Akt = phosphorylated Akt; p-ERK = phosphorylated

extracellular signal-regulated kinase; p-GSK-3 β = phosphorylated glycogen synthase kinase-3 β ;

p-IGF-1R = phosphorylated insulin-like growth factor-1 receptor; p-JNK = c-Jun NH2-terminal kinase;

p-STAT3 = phosphorylated signal transducer and activator of transcription 3; P-gp = P-glycoprotein

efflux pump; TF = theaflavin; TFGA = theaflavin-3-gallate; TFGB = theaflavin-3'-gallate; TFDG =

theaflavin 3,3'-di-O-gallate; Topo-II = topoisomerase II; VEGFR-2 = vascular endothelial growth factor

receptor-2.

Table 7. Biological effects of oolong tea in human case studies of cancer.

Type of cancer	Compound(s)	Model(s) [Test/Control]	Biological end point	Effective dose	Reference
Head and Neck Cancer (HNC)	oolong tea extract	Both men and women [396/413]	↓ the risk of HNC	150-450ml per day	Huang, <i>et al.</i> , 2014
Ovarian Cancer	oolong tea extract	Both men and women [134/216]	↓ the risk of ovarian cancer	180ml per day	Lee <i>et al.</i> , 2013
Esophageal Squamous Cell Carcinoma (ESCC)	tea catechins	Men only [487/755]	↓ the risk of ESCC	>300 units of catechins per day	Chen <i>et al.</i> , 2009
Colorectal Cancer	oolong tea extract	Men only [162/806]	↓ risk of colorectal cancer	Dependent	Yuan <i>et al.</i> , 2007

Table 8. Caffeine content (in mg) of different tea drinks per 8 fl.oz (240 ml) cup.

Teas	Caffeine content (mg)
Green Tea	15-35
Oolong Tea	15-50
White Tea	10-25
Black Tea	14-61 (on average, 40)

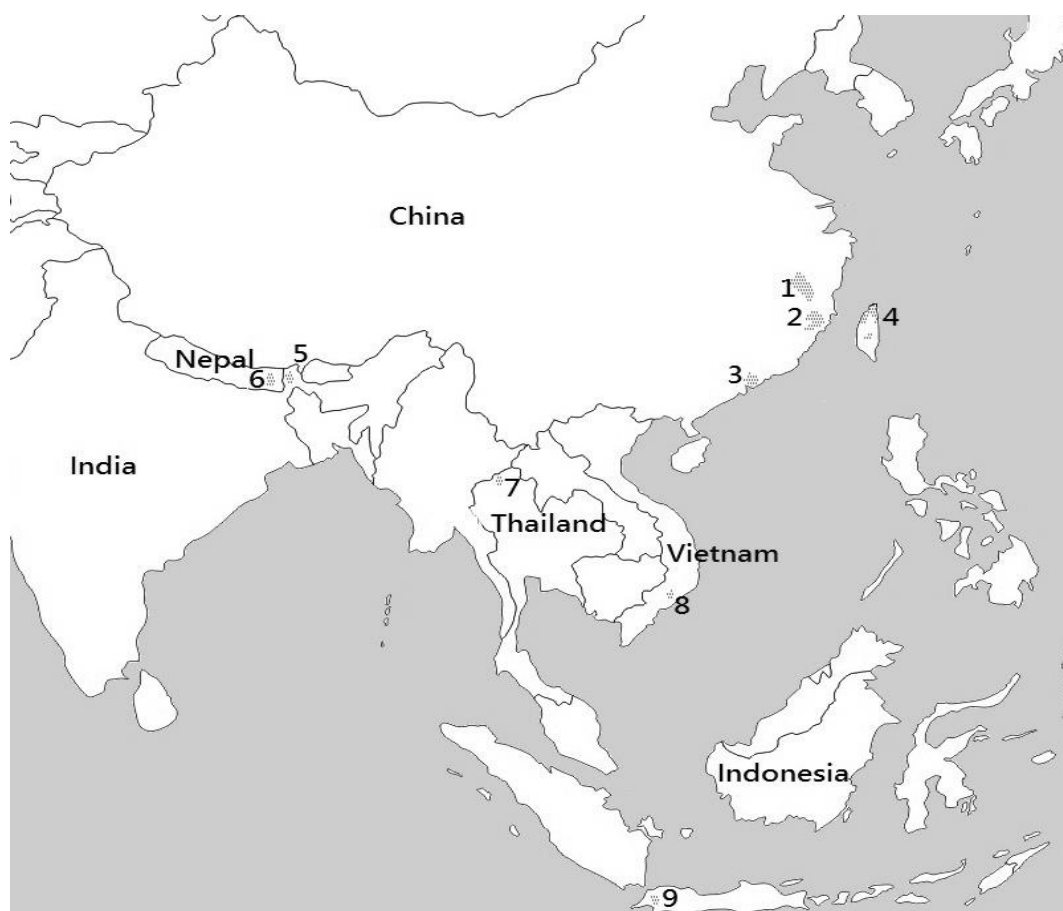


Figure 1. Distribution of oolong tea production in Southeast Asia. Wuyi Mountain (1) in north Fujian; Anxi (2) in south Fujian; Phoenix Mountain (3) in Guangdong; Taipei, Hsinchu, Yilan and Nantou (4) in Taiwan; Darjeeling (5) in India; Ilam, Panchthar and Dhankuta (6) in Nepal; Mae Salong (7) in Thailand; Lam Dong (8) in Vietnam and Lebak-Banten (9) in Indonesia.

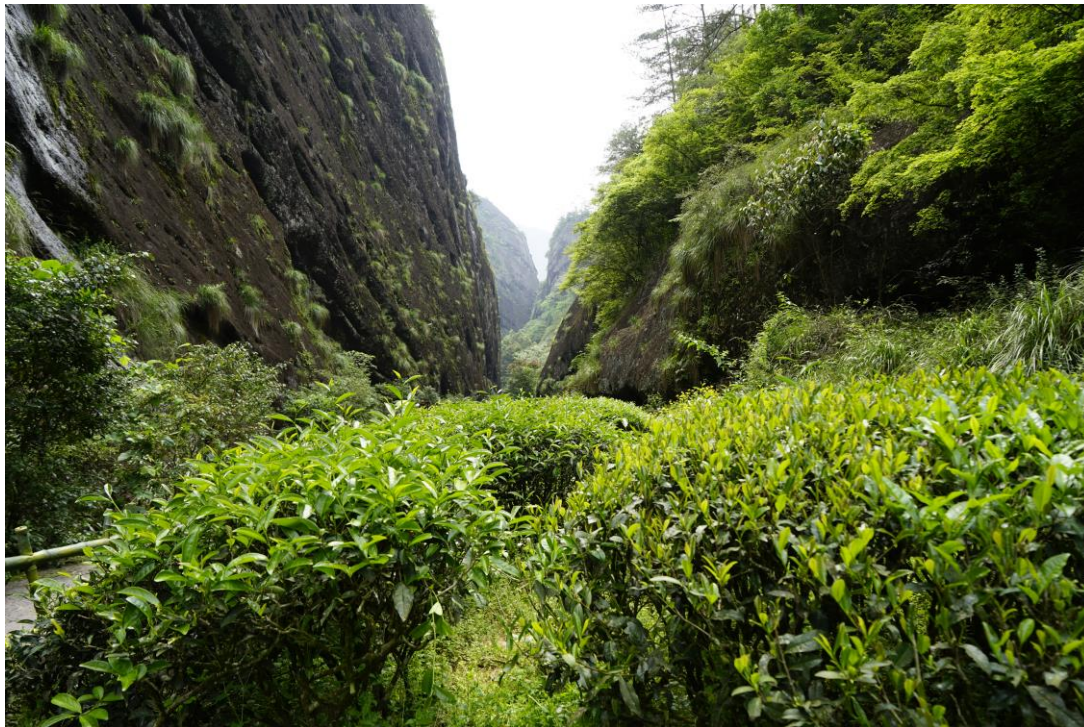


Figure 2. Growing condition of "rock teas" in the Wuyi Mountains, Fujian, China.

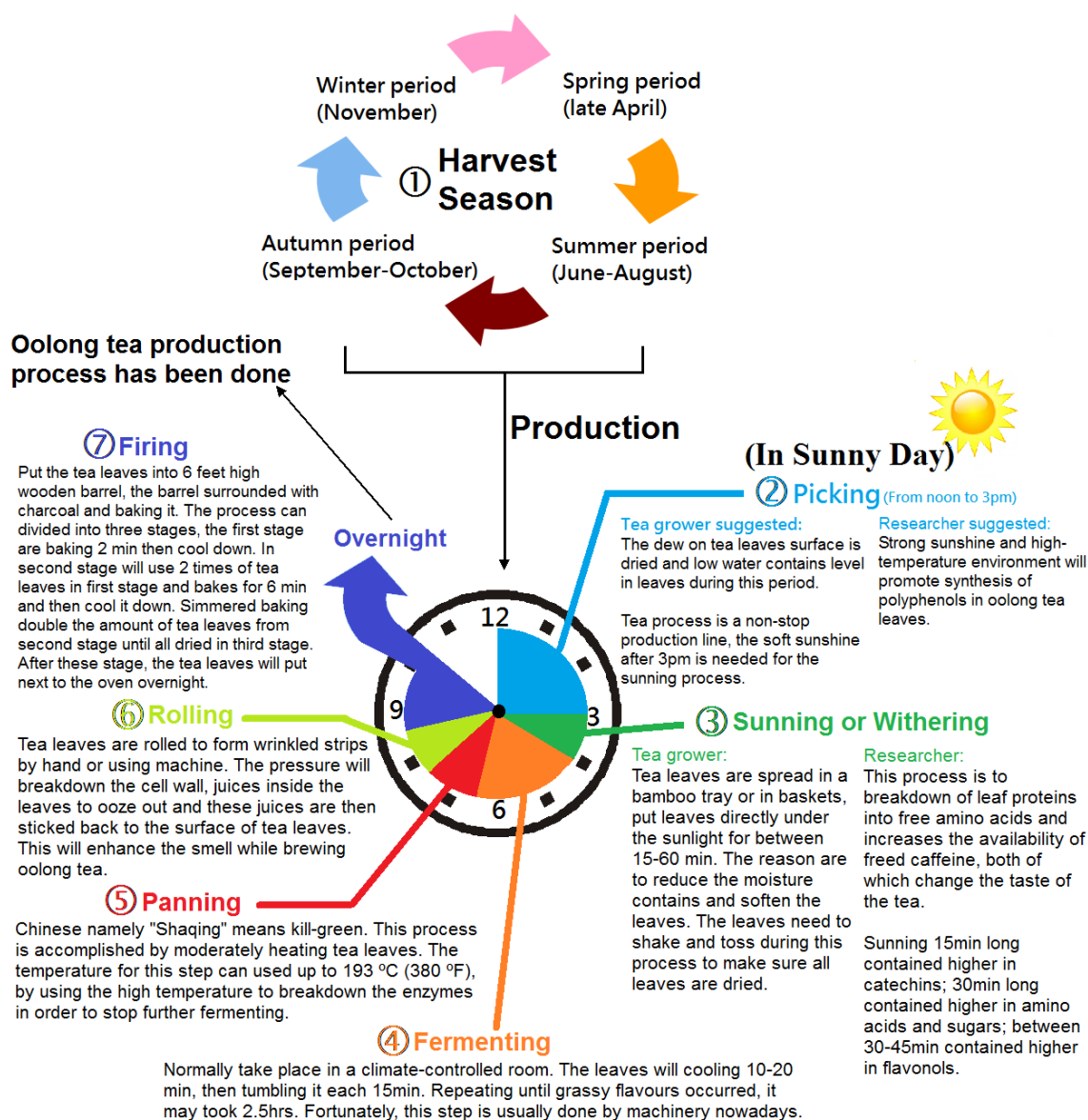


Figure 3. Production of oolong tea.



Figure 4. A basic "Kung-Fu tea" set, tools and equipment namely hot water kettle (1), brewing tray (2), tea cups (3), brewing vessel (4), tea pitcher (5), tea towel (6), tea tongs (7), tea leaf holder (8), tea basin for used tea leaves and refuse water (9) and oolong tea (10).

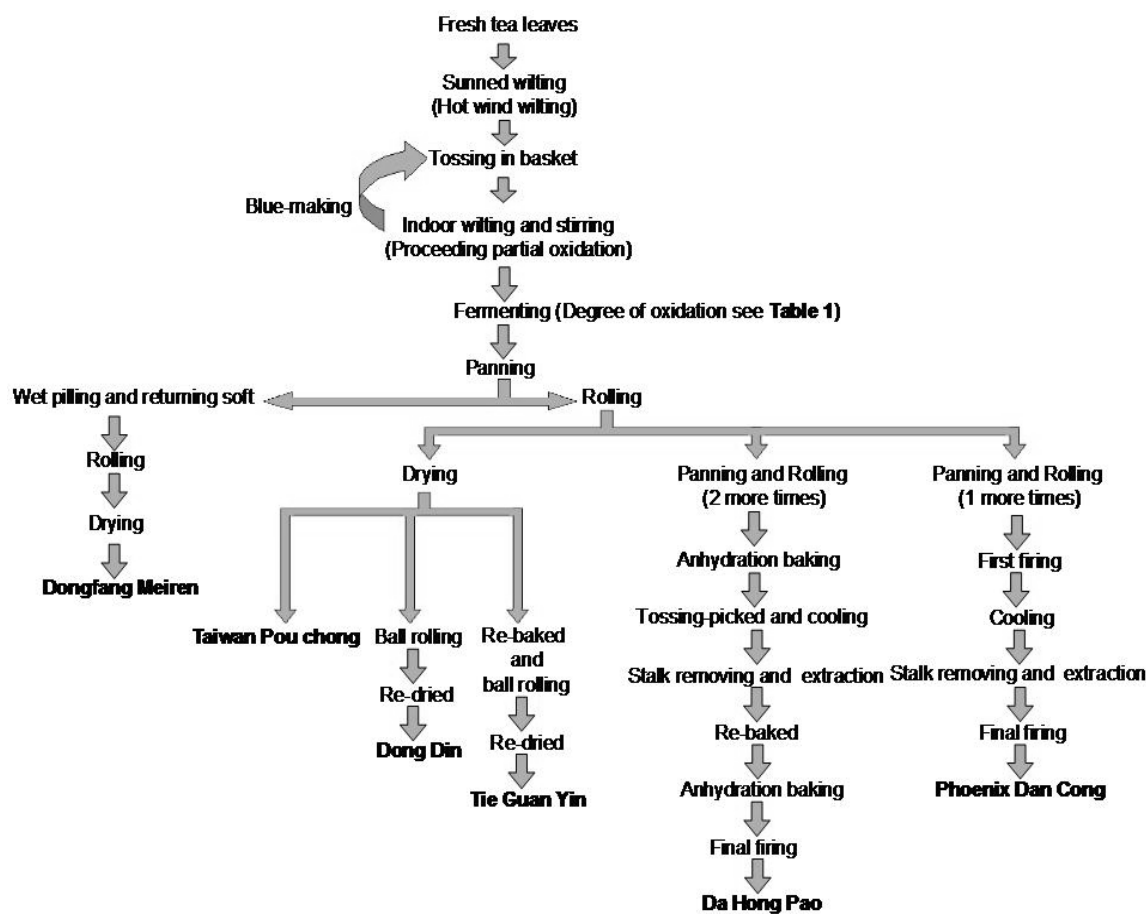


Figure 5. Comparison of the preparation for six varieties of oolong tea.

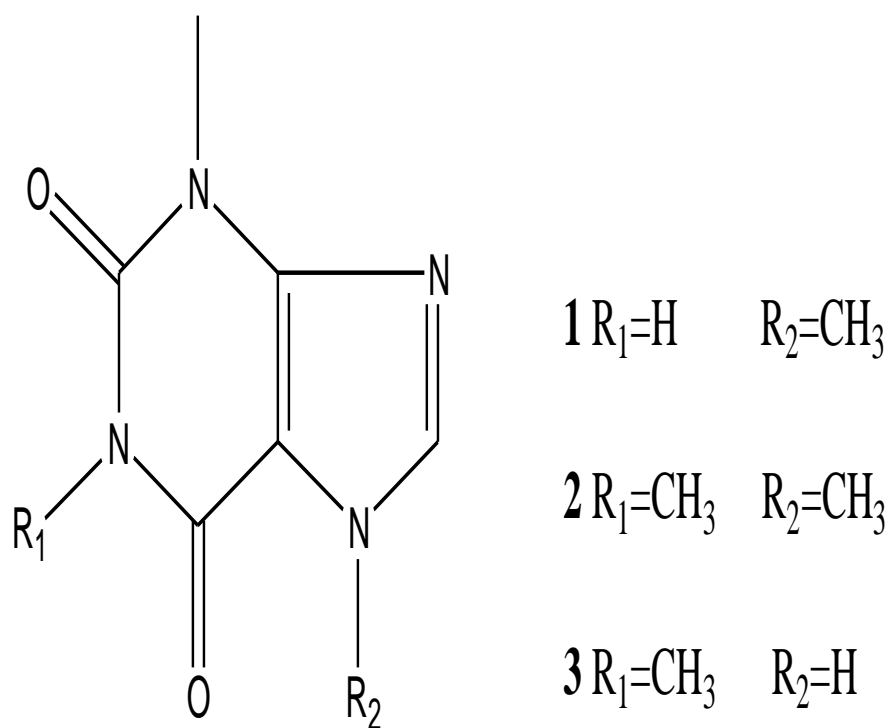


Figure 6. Structures of alkaloids in oolong tea.

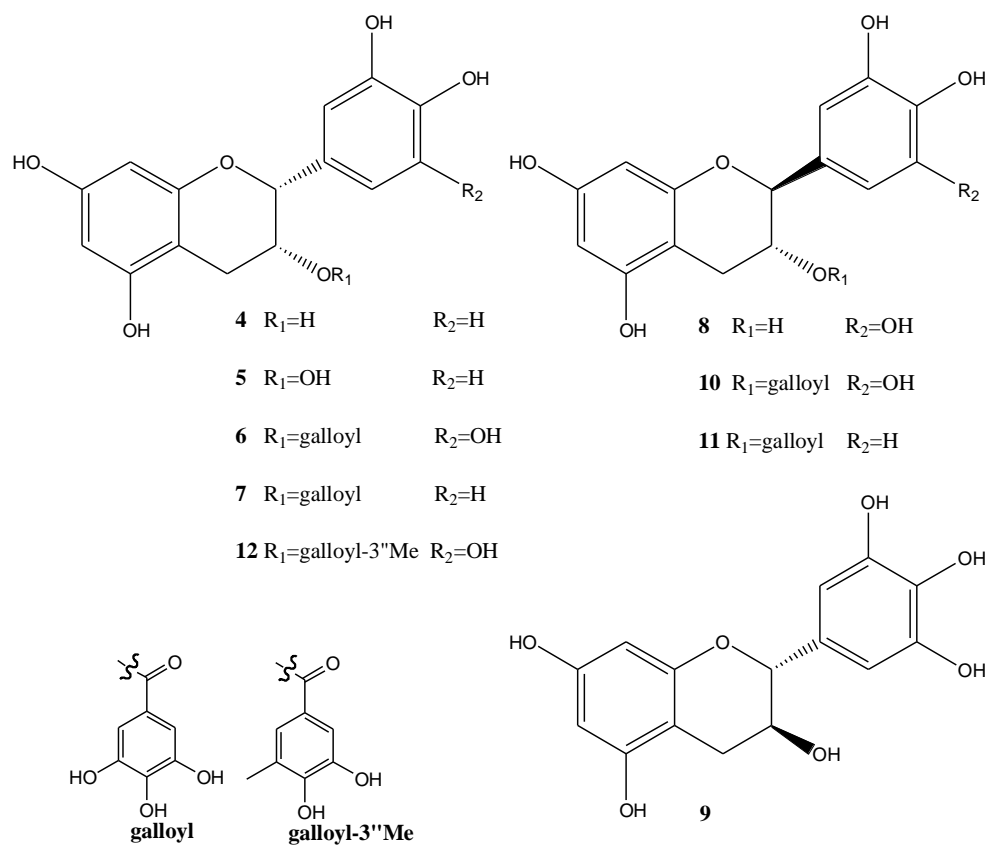


Figure 7. Structures of catechins in oolong tea.

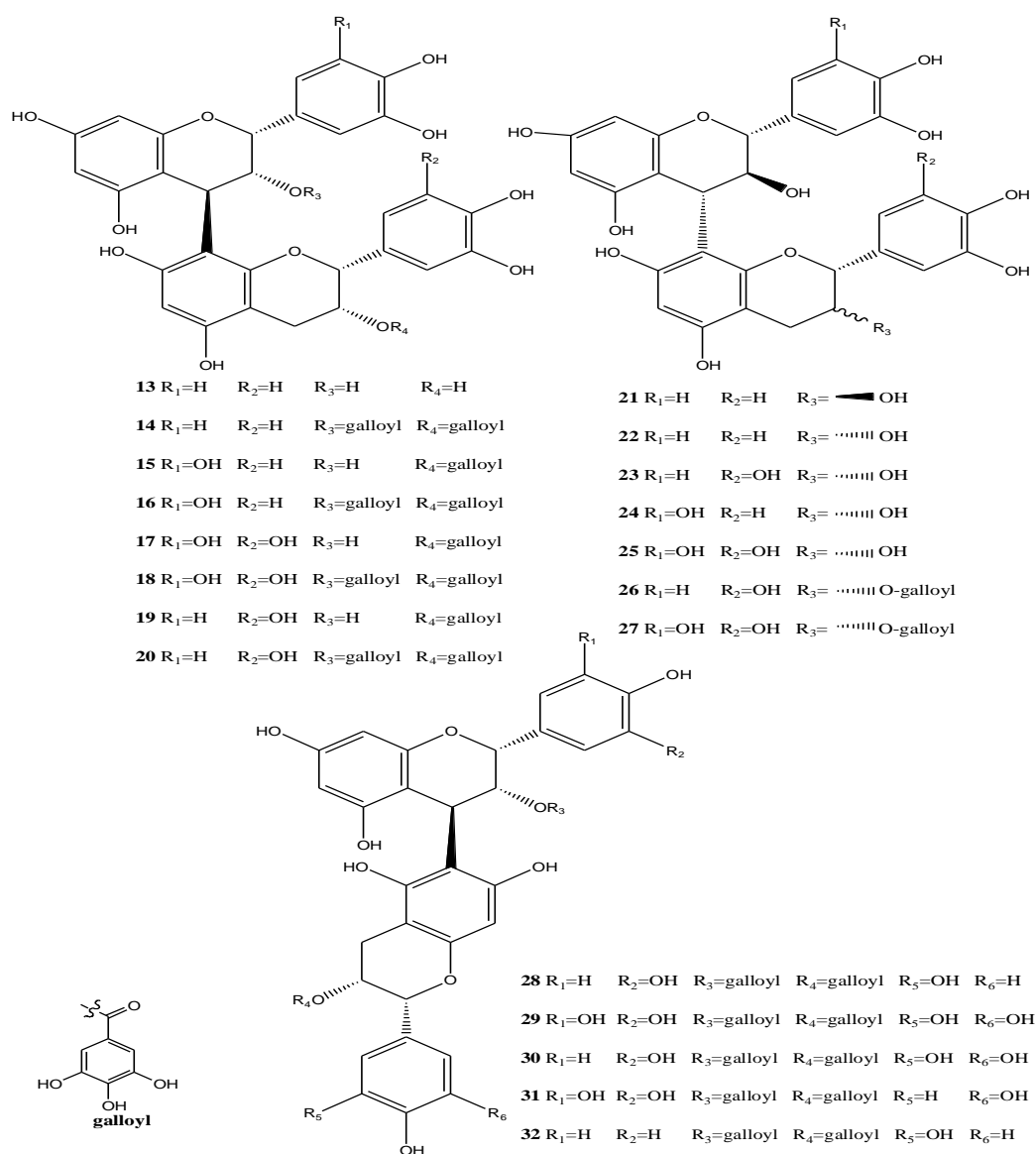


Figure 8. Structures of proanthocyanidina-type tannins in oolong tea.

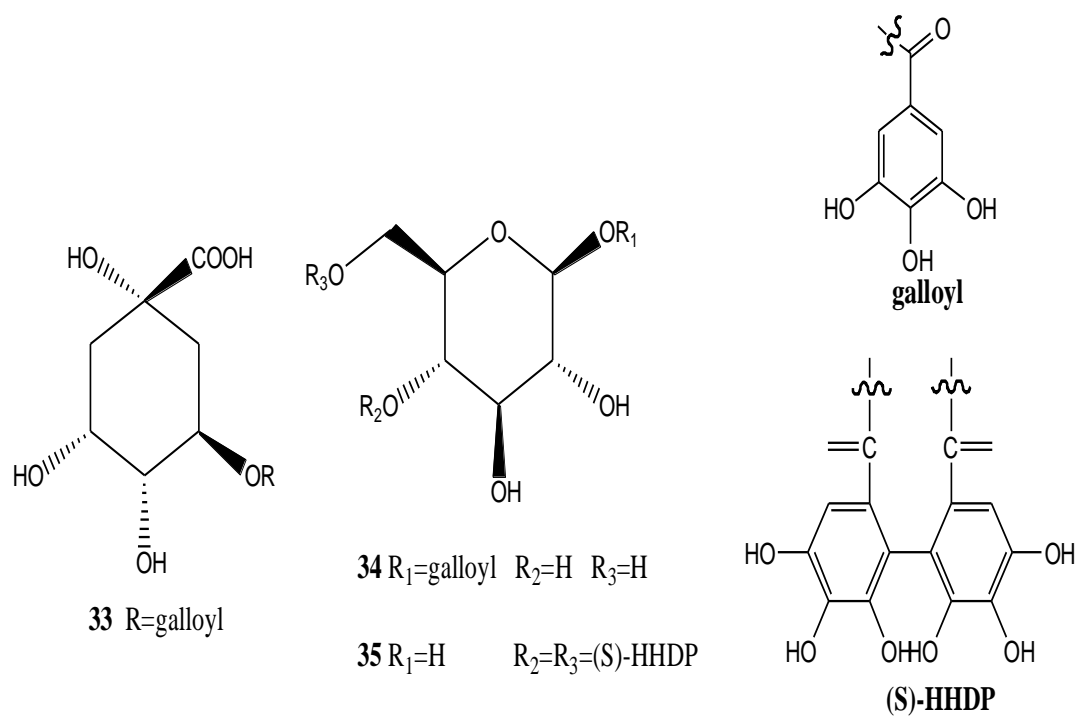


Figure 9. Structures of hydrolyzable tannins in oolong tea.

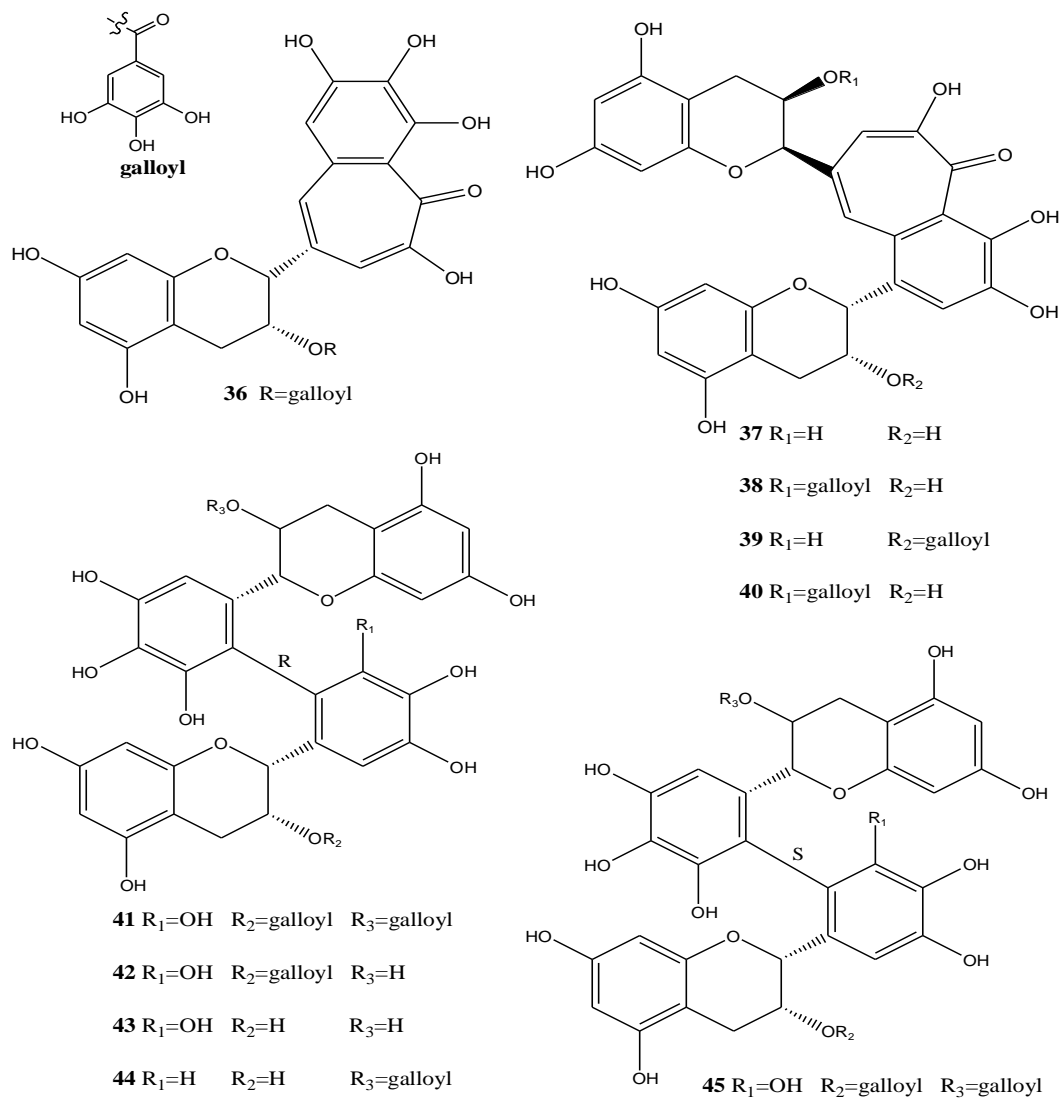


Figure 10. Structures of tea pigments in oolong tea.

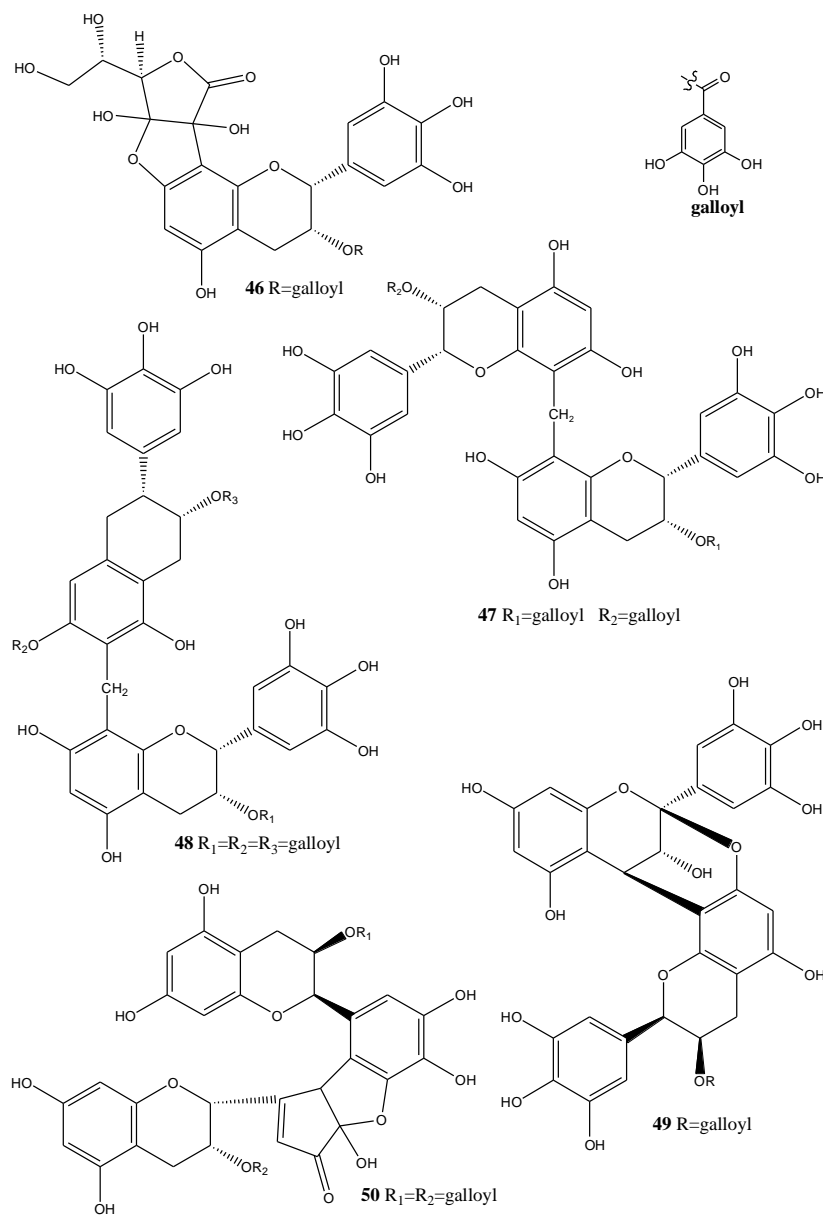


Figure 11. Structures of other tannins in oolong tea.

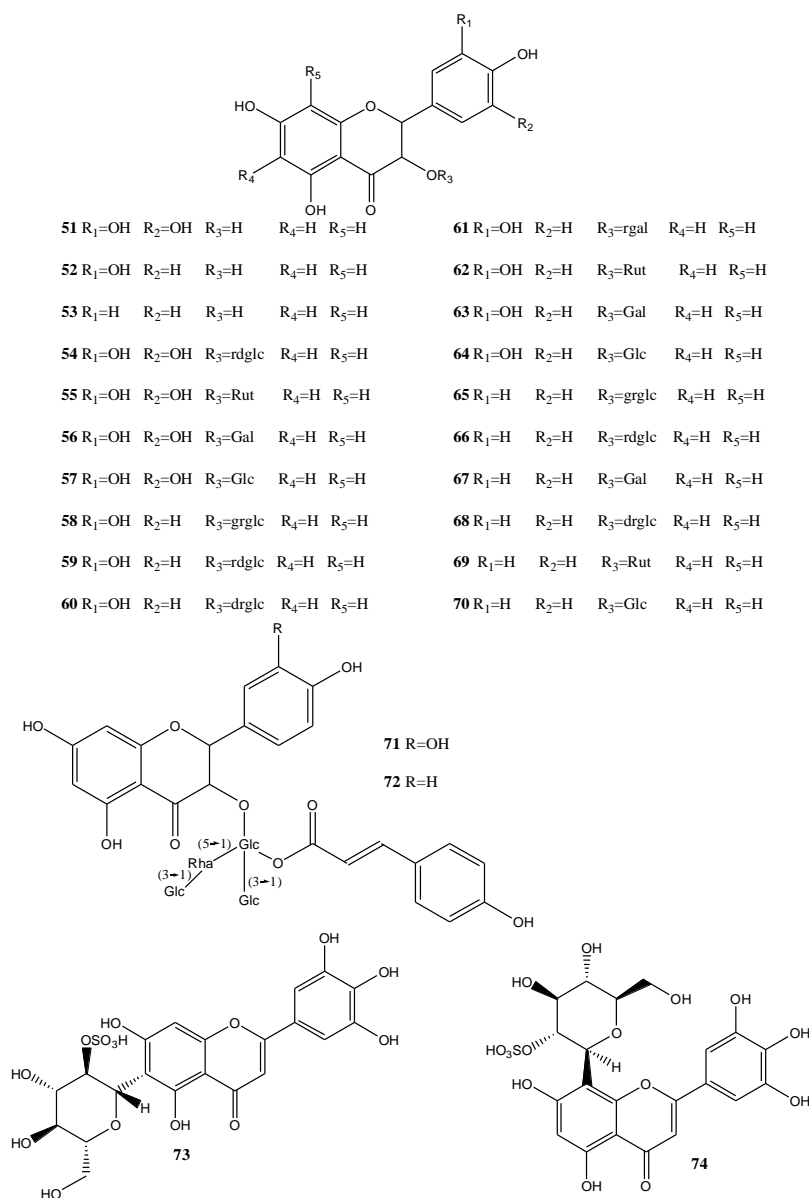


Figure 12. Structures of flavonol and flavonol glycosides in oolong tea.

drglc=dirhamnoglucoside; Gal=galactoside; Glc=glucoside; grgic=glucorhamnoglucoside;

rdglc=rhamnoglucoside; rgac=rhamnoglactoside; Rha=rhamnosidase; Rut=rutinoside.

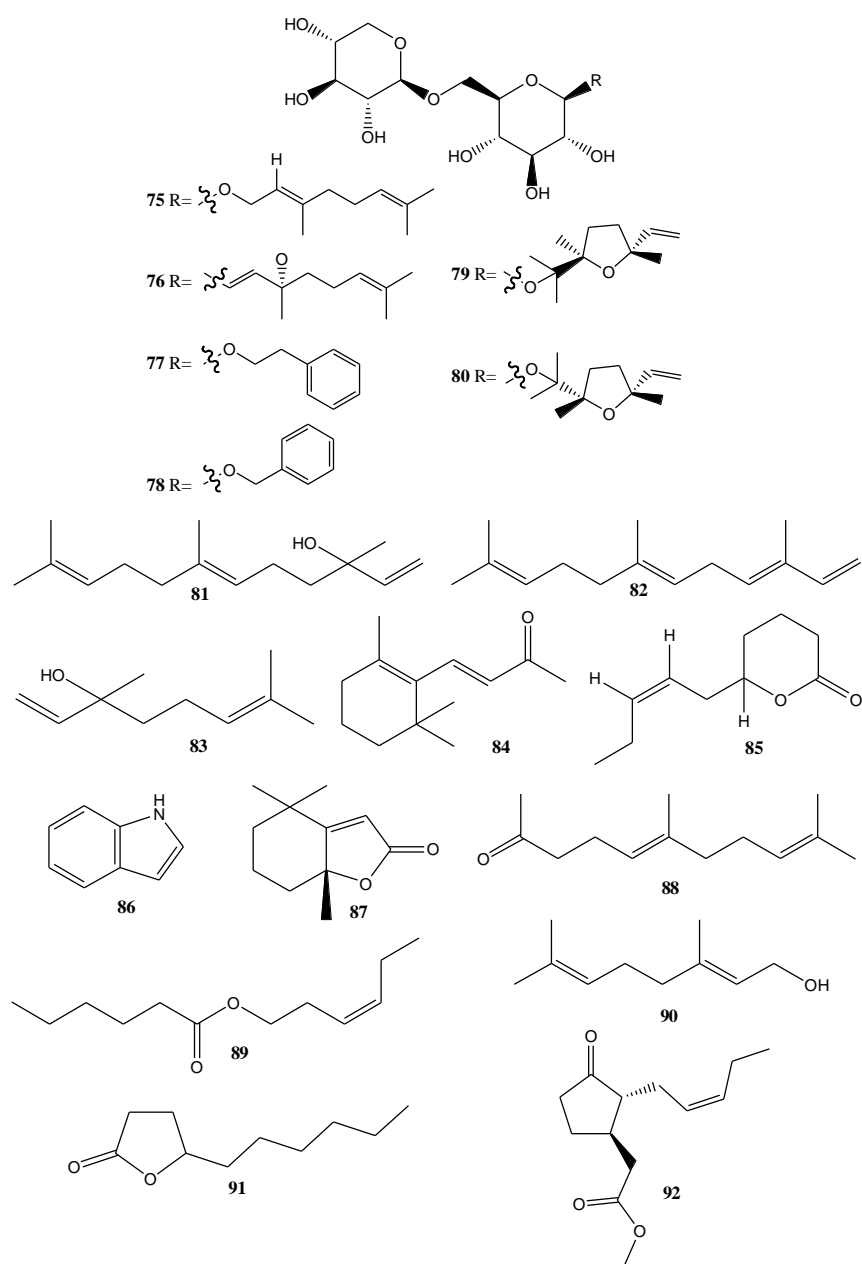


Figure 13. Structures of aromas in oolong tea.

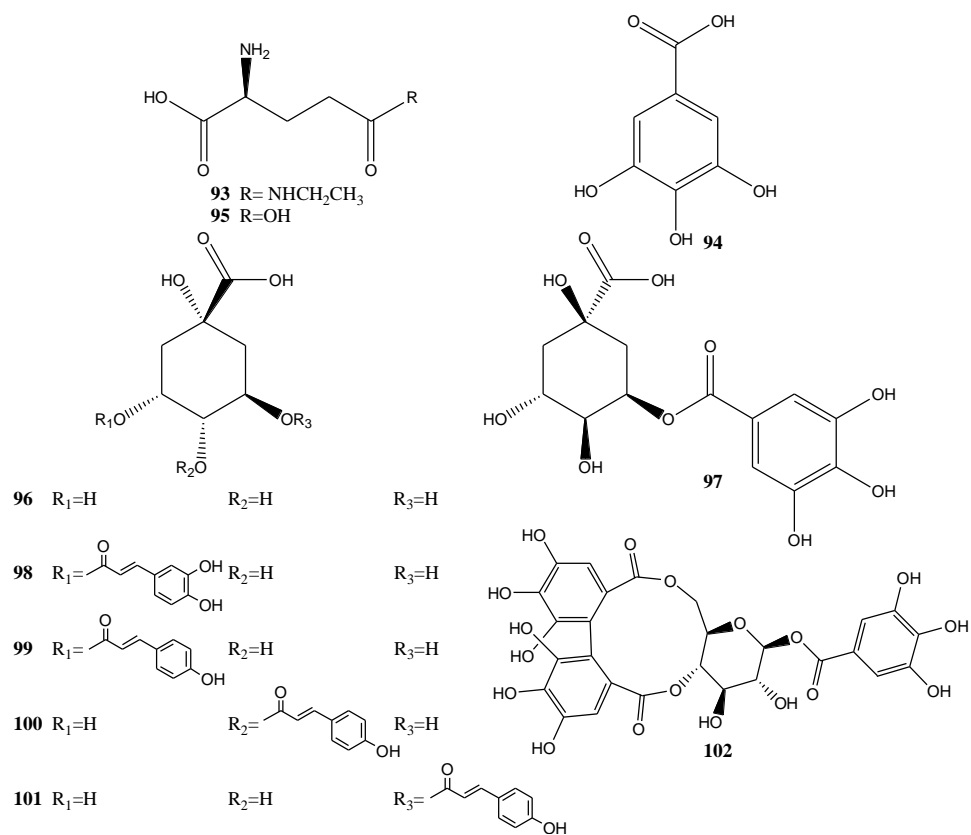


Figure 14. Structures of amino acids and organic acids in oolong tea.

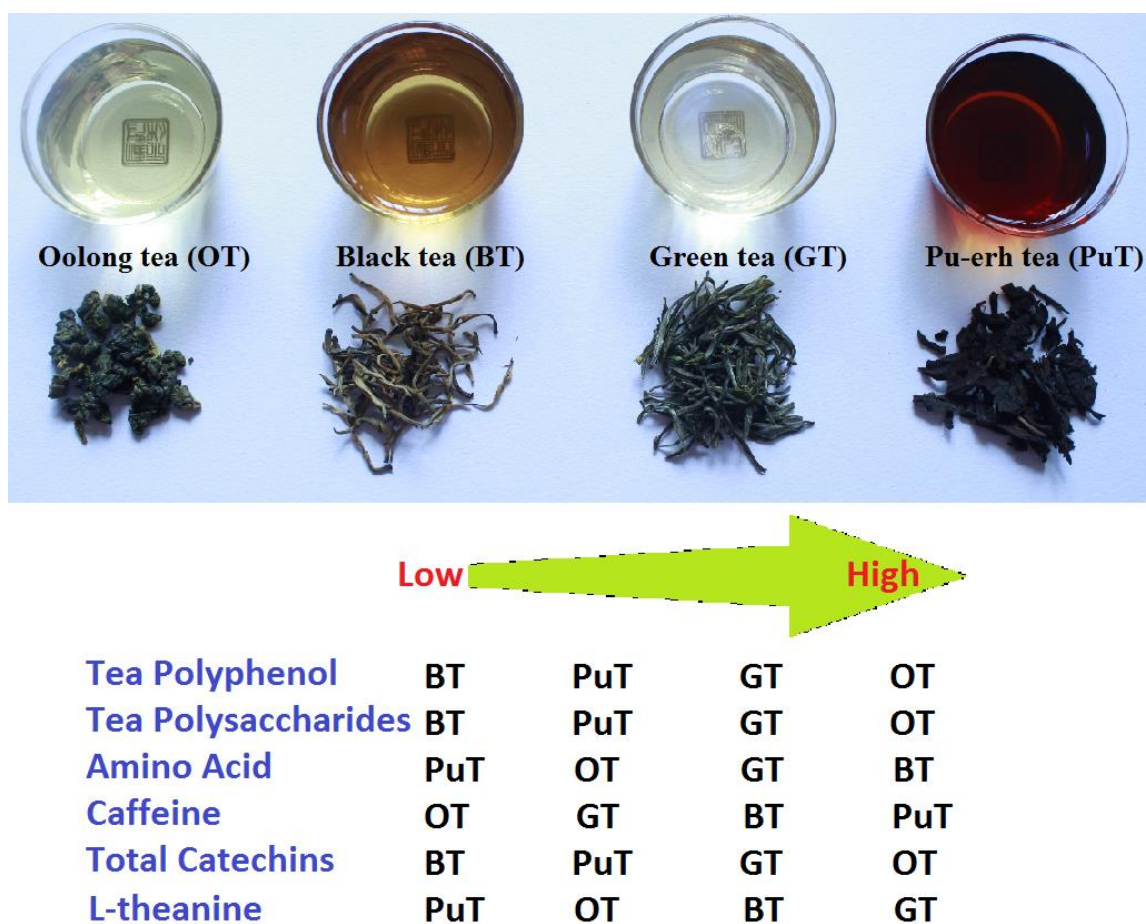


Figure 15. The distinctions of chemicals contain in different tea types.

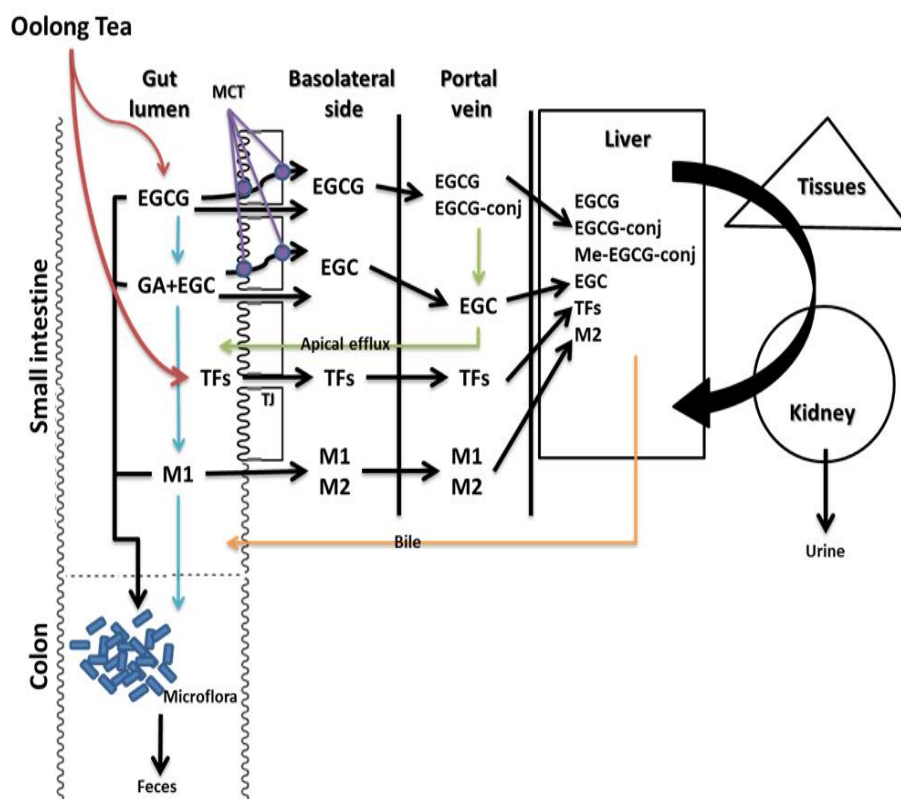


Figure 16. The outline of a possible metabolism route of oolong tea. EGCG = epigallocatechin gallate, EGC = epigallocatechin, EGCG-conj = EGCG conjugates, Me-EGCG-conj = methylated EGCG conjugates, gallic acid = GA, M1 = 5-(3',5'-dihydroxyphenyl)- γ -valerolactone, M2 = 5-(5'-hydroxyphenyl)- γ -valerolactone-3'-O- α -glucuronide, MCT = monocarboxylic acid transporter, TFs = theasinensins, TJ = tight junction.

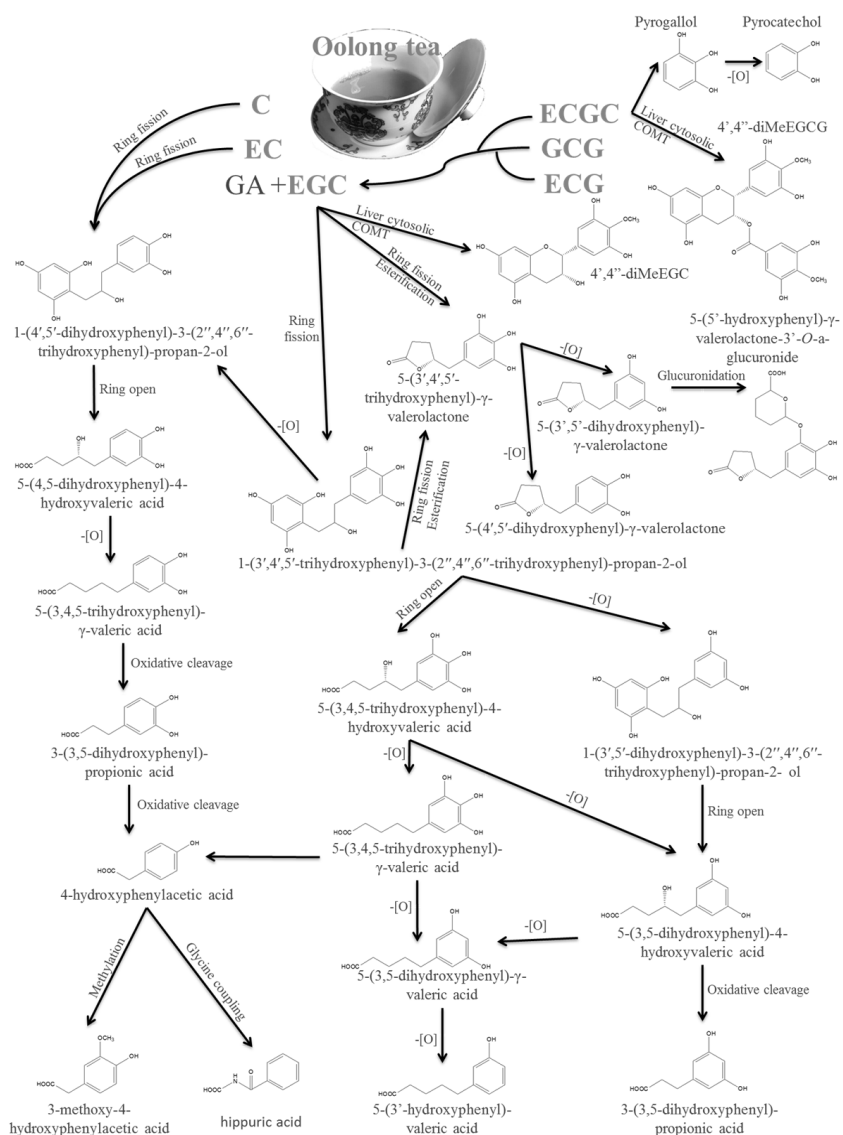


Figure 17. The *in vitro* and *in vivo* biotransformation of catechins. C = catechin, EC = epicatechin, ECG = epicatechin gallate, ECGC = epigallocatechin gallate, EGC = epigallocatechin, GA = gallic acid, GCG = galocatechin gallate.