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Stability and chemical changes of phenolic compounds during Oolong tea processing

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Abstract

A study was conducted to investigate the impact of lightly fermented Oolong tea processing on the alteration of constituents, particularly phenolic compounds. Harvested tea shoots were immediately processed under strict processing control. Tea infusions brewed with each process unit operation were analyzed for total polyphenols, caffeine (CF), gallic acid (G) and catechin profiles. During processing, the contents of total polyphenols and CF significantly remained constant, while G significantly increased. The content of total catechins was slightly decreased, approximately 10% during the manufacture of Oolong tea from fresh tea shoots. Among the process unit operations, indoor withering, pan-firing and drying steps had impact on catechin concentrations. This result should be considered for tea manufacturing in order to maximize potential health benefits from catechins in Oolong tea.

Introduction

Tea is one of the most widely consumed beverages in the world, and it is recognized for its high content of polyphenols, which have received a great deal of attention due to their many health benefits (Grant and Dworakowska, 2013; He *et al*., 2013; Kapiszewska *et al*., 2013; Pan *et al.*, 2013; Riegsecker *et al*., 2013; Sharma, 2014). Most of the processed tea produced in the world is made from the young tender shoots (flushes) of *Camellia sinensis* (L.) O. Kuntze and can be classified on the basis of the fermentation as non-fermented tea (green tea), semi-fermented tea (Oolong tea) and fully-fermented tea (black tea).

Green, Oolong and black contain high amount of polyphenols in different compounds due to fermentation. Green tea contains significant quantities of the unoxidized catechins: (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin gallate (EGCG), (-)-gallocatechin gallate (GCG), (-)-epicatechin gallate (ECG) and (-)-catechin gallate (CG); the oxidized derivatives of the catechins, theaflavins (TFs) and thearubigins (TRs), are found in fully-fermented (black) and semi-fermented (Oolong) teas (Peterson *et al*., 2005; Stodt and Engelhardt, 2013). Catechins are the highest in concentration in green teas, while Oolong and black tea has substantially fewer due to its oxidative production. Catechins are the major active component representing up to 30% of the dry weight of green tea leaves while caffeine is the second major component.

Because the health benefit of polyphenols in teas, tea manufacturers try to produce the tea product with the high content of polyphenols. However, the content and stability of polyphenols during tea manufacturing depend on many factors such as cultivar, harvest season, age of the plant, climate, environmental conditions and processing conditions (Owuor *et al.*, 2008). Among these factors, processing condition is one of the most importances that determine the quality of the tea product. Many studies have been focused on the effect of processing steps on the content of polyphenols, mainly in green tea (Namal Senanayake, 2013; Shitandi *et al*., 2013; Tontul *et al.*, 2013; Wachira *et al.*, 2013; Bruno *et al*., 2014; Kaur *et al*., 2014; Kosińska and Andlauer, 2014; Monsanto *et al.*, 2014) and black teas (Senthil Kumar *et al.*, 2013; Dutta and Baruah, 2014; Kosińska and Andlauer, 2014; Maria John *et al*., 2014). There are little studies focused on Oolong teas (Chen *et al*., 2010; Chen *et al*., 2013; Chen *et al*., 2014). This may be because the most tea consumed is black tea (78%) followed by green tea (20%), while Oolong tea is consumed only 2% worldwide. Although Oolong tea is now getting more and more popular in the world, there is much less investigation in comparison with the vigorous studies on the active ingredients found

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in green and black teas.

The major chemical constituents of Oolong tea are polyphenols, caffeine, amino acids, protein, and chlorophyll. Among these chemicals, polyphenols are well-studied bioactive components. The phenolic compounds identified in Oolong tea are (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), (+)-catechin (C), (-)-gallocatechin (GC), (-)-catechin gallate (CG), and (-)-gallocatechin gallate (GCG). Chemical structures of the catechins as well as gallic acid (G) and caffeine (CF) are shown in Figure 1.

These catechins are regarded as the most important, due to its high content in tea and its health benefits (Singh *et al*., 2011). The identification and determination of catechins have been focused for the chemical control of Oolong tea products (Horanni and Engelhardt, 2013; Kausar *et al.*, 2013). Therefore, tea manufacturers try to produce the Oolong tea with the high content of catechins to respond to the increasing market for functional foods having potential health benefits. However, the content and the stability of catechins depend on many factors during processing (Shitandi *et al*., 2013; Kaur *et al*., 2014).

Tea catechins undergo many chemical changes during the course of the manufacturing and brewing processes. They are very prone to epimerization and degradation reactions (Sang *et al.*, 2011; Ananingsih *et al.*, 2013; Li *et al*., 2014). These reactions are undesirable, especially in commercial tea drinks where only little amount of catechins was found (Labbé *et al*., 2008). As a result, many studies have been done to investigate the stability of catechins in aqueous solutions during processing and storage (Bazinet *et al*., 2010; Sang *et al.*, 2011; Li *et al.*, 2012; Ananingsih *et al*., 2013) not in tea leaves during processing. Little information is available on the changes of catechins during Oolong tea processing, especially when the starting fresh tea shoots are the same and the Oolong tea process was strictly controlled by the same expert. To produce a high quality Oolong tea with high content of catechins, it is important to understand the stability of phenolic compounds during Oolong tea processing in order to gain the optimum health benefits from them. In this study, the main objectives were to (1) explore process unit operations of Oolong tea at a tea factory, (2) study the changes of catechins during processing, and finally (3) evaluate the impact of the different process unit operations on the changes of catechins in Oolong tea production in Thailand.

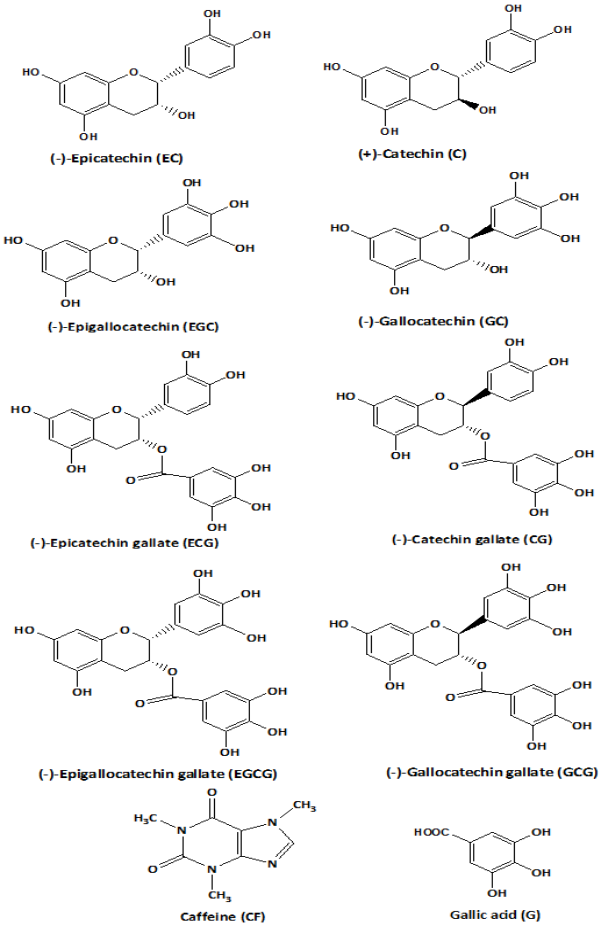

Figure 1. Chemical structures of catechins, caffeine and gallic acid

Materials and Methods

Chemicals and reagents

The standards , which include gallic acid (G, ≥ 99%), (-)-gallocatechin (GC, ≥ 98%), (-)-epigallocatechin (EGC, ≥ 98%), (+)-catechin (C, ≥ 98%), (-)-epicatechin (EC, ≥ 90%), (-)-epigallocatechin gallate (EGCG, ≥ 95%), caffeine (CF, ≥ 99%), (-)-gallocatechin gallate (GCG, ≥ 98%), (-)-epicatechin gallate (ECG, ≥ 98%) and (-)-catechin gallate (CG, ≥ 98%), were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Folin–Ciocalteu's phenol reagent, acetonitrile, trifluoroacetic acid (TFA) and methanol (HPLC-grade) were purchased from Fluka (Buchs, Switzerland). Anhydrous sodium carbonate was purchased from Merck (Darmstadt, Germany). Water was purified with a Milli-Q water purification system (Millipore, Bedford, USA). All other chemicals and solvents in this study were of analytical grade.

A survey of Oolong tea processing

A survey of Oolong tea processing was conducted at the tea garden and tea factory of Thai Tea Suwirun, Mae Lao District, Chiang Rai Province, Thailand.

Process unit operations of Oolong tea were surveyed before the experiment was started.

Collection of samples

The leaves of the Chinese tea (*Camellia sinensis* var. *sinensis*, Jin Xuan Oolong or Oolong no.12 cultivar), grown in the same location, were harvested, comprising a bud and two leaves. Fresh tea shoots were divided into three groups and immediately processed into a lightly fermented Oolong tea (degree of fermentation about 10-20%). The tea leaves (250 g) were collected after each processing step, including plucking, outdoor withering, indoor withering, pan-firing, rolling and drying. Samples were immediately vacuum-packed in polypropylene plastic bags and stored at -20˚C for approximately 3-5 days prior to analysis. During the period of sample collection, three samples were sampled in each step.

Determination of moisture content

Tea samples, ~5 g, weighed to the nearest 0.001 g, were placed in a moisture can and heated in an oven at 103±2˚C for at least 16 h to constant weight. The percentage of moisture content (%MC) and dry matter (%DM) in the samples were then calculated from the weight differences. All tests were performed in triplicate.

Sample extraction

Tea leaves collected from processing steps were differently weighed due to the difference in moisture content of tea leaves in each processing step. For tea leaves collected from plucking, outdoor withering, indoor withering, pan-firing, rolling and drying steps, about 10, 10, 10, 5, 2 and 2 g (weighed to the nearest 0.001 g) were weighed, respectively. Then, tea samples were finely ground into a powder with a blender or a homogenizer and were extracted with 200 mL of distilled water at 80°C for 15 min. The extraction mixture was filtered through a filter paper (Whatman No. 4). The residue was washed with distilled water (3x10 mL). The tea solution was cooled to room temperature and adjusted to 250 mL with distilled water. All samples were extracted in triplicate.

Determination of total polyphenol content (TPC)

The total polyphenol content (TPC) was determined by spectrophotometry, using gallic acid as a standard, according to the method described by the International Organization for Standardization (ISO 14502–1, 2005). Sample extracts were diluted (50-fold) with distilled water and 1.0 mL portions of the diluted solution were transferred in duplicate to separate tubes containing 5.0 mL of 10%v/v dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 mL of sodium carbonate solution (7.5% w/v) was added with mixing. The tubes were then left to stand at room temperature for 60 min before absorbance at 765 nm was measured. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10-100 µg/mL. The TPC was expressed as gallic acid equivalents (GAE), i.e. GAE g/100 g dry weight (DW).

Determination of individual catechins, total catechins, gallic acid and caffeine

Individual catechins, total catechins content (TCC), gallic acid (G) and caffeine (CF) were determined by ISO method (ISO 14502–2, 2005) with slight modifications. The individual standard solutions of G, GC, EGC, C, EC, EGCG, GCG, ECG, CG and CF were prepared by dissolving them in methanol to generate a stock concentration of 1,000 µg/mL. The mixed stock standard solution was prepared by mixing an equal volume of each stock standard. Working standard solutions were prepared by dilution of the mixed stock solution and then filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter (Millipore Ltd., Bedford, USA. HPLC analysis of standards and sample extracts (10-fold dilution with distilled water) was conducted on Water 966 high performance liquid chromatography, comprising vacuum degasser, quaternary pump, auto-sampler, thermostatted column compartment and photo diode array detector. The column used was a Platinum EPS C18 reversed phase, 3 µm (53x7 mm) with the column temperature of 30°C. Mobile phase was water/acetonitrile (87:13) containing 0.05% (v/v) trifluoroacetic acid (TFA) with the flow rate of 2 mL/min. The injection volume was 20 µL. Absorption wavelength was 210 nm. Validation was carried out in compliance with the AOAC International guidelines for single Lab validation of chemical methods for dietary supplements and botanicals (AOAC, 2002). Individual catechins, gallic acid and caffeine were identified by comparing their retention times and UV spectra in the 190-400 nm range with standards. Calibration graphs were constructed using nine levels of concentration which covered the concentration ranges expected in the tea samples. The linearity of the HPLC method was checked for gallic acid, caffeine and individual catechins (0.20-100.0, 0.10-51.9, 0.09-43.1, 0.20-100.0, 0.19-97.0, 0.16-80.0, 0.20-100.0, 0.11-52.9, 0.20-98.0, 0.09-44.1 µg/mL for G, GC, EGC, C, EC, EGCG, CF, GCG, ECG and CG, respectively. All the analytes exhibited good linearity (r) over the range tested with correlation coefficient from 0.9965 for

GCG to 0.9997 for CF. Individual catechins, gallic acid and caffeine were quantified using calibration curves. The results were expressed as g/100 g DW. The total catechin content (TCC) as g/100 g DW was determined by the summation of individual catechins (GC, EGC, C, EC, EGCG, GCG, ECG and CG).

Statistical analysis

Data were expressed as means ± standard deviation. The data were also subjected to analysis of variance (ANOVA) and Duncan's multiple range tests using SPSS 16.0 for Windows. The significance level of $P<0.05$ was considered significantly different.

Results and Discussion

A survey of Oolong tea processing

Oolong tea processing in Thailand consisted of 6 processing steps, including plucking, outdoor withering, indoor withering, pan-firing, rolling and drying (Figure 2). All Oolong tea processes were controlled by experts during the production, and the final fermentation degree of Oolong tea was 20%. In the production of Oolong tea, young tea shoots (one bud and two leaves) were plucked by hand and withered in direct sunlight (outdoor withering) to remove the moisture for about 8 to 12 hours. During the exposure to sunlight, leaves were turned 2–3 times. When the tea shoots became soft and the total moisture lost, the tea shoots were moved to indoor withering in a room with the control at a temperature of 20–25°C and at a relative humidity of 75–85%. During indoor withering step, the tea shoots were rotated by a special bamboo machine about 4-6 times. The rotating of leaves caused damage to leaf edges and fermentation occurred. After the desired oxidation level was reached (20%), the leaves were pan-fired at high temperatures (about 250-300˚C) for about 5-10 min to prevent further oxidation. Next, the leaves were rolled using a rolling mill. The tea leaves were then wrapped up and tumbled to give the characteristic of ball shape. After ball-shaping, the leaves were dried by the drying machine. Finally, the Oolong teas were sorted, graded, and packaged.

Changes in moisture content and total polyphenols

Fresh tea shoots had an average moisture content of 76.29±3.59 g/100 g (Table 1). The moisture content of fresh tea shoots decreased gradually from the plucking step to the indoor withering step and dramatically decreased in the pan-firing step. The average moisture content of Oolong tea after drying was 2.07±0.20 g/100 g. The one-way analysis of variances of the moisture content showed that there were significant differences in moisture contents in all steps of Oolong tea processing.

Figure 2. Processing steps of Oolong tea

The result of the Folin-Ciocalteu assay showed that the fresh tea shoots contained 15.17±1.22 GAE g/100 g DW and the Oolong tea contained 14.27±1.03 GAE g/100 g DW (Table 1). The total phenolic content of the Oolong tea was slightly lower than that of the fresh tea shoots. However, the one way analysis of variance demonstrated that the total polyphenols contents were not significant differences in all steps of Oolong tea processing. It indicated that the concentrations of total polyphenols were not affected by the processing. It should be noted that the Folin-Cioclateu assay is a redox method based on the ability of phenolics to react with oxidizing agents. Usually, redox assays are affected by the varying hydroxylation pattern, the degree of oxidation, and the degree of polymerization of polyphenolics. The unaltered total phenolic content might be due to the fact that the phenolic compounds were softly oxidized or polymerized. Moreover, they might be transformed via the epimerization and degradation during Oolong tea processing.

Changes in HPLC chromatograms

Figure 3 shows HPLC chromatograms of the authentic standards of gallic acid, caffeine and eight catechins (A), fresh tea shoots (B) and Oolong tea (C). It clearly shows that there was a difference in the HPLC chromatograms between the beginning and the end of the Oolong tea processing. Originally, gallic acid, caffeine and seven catechins were

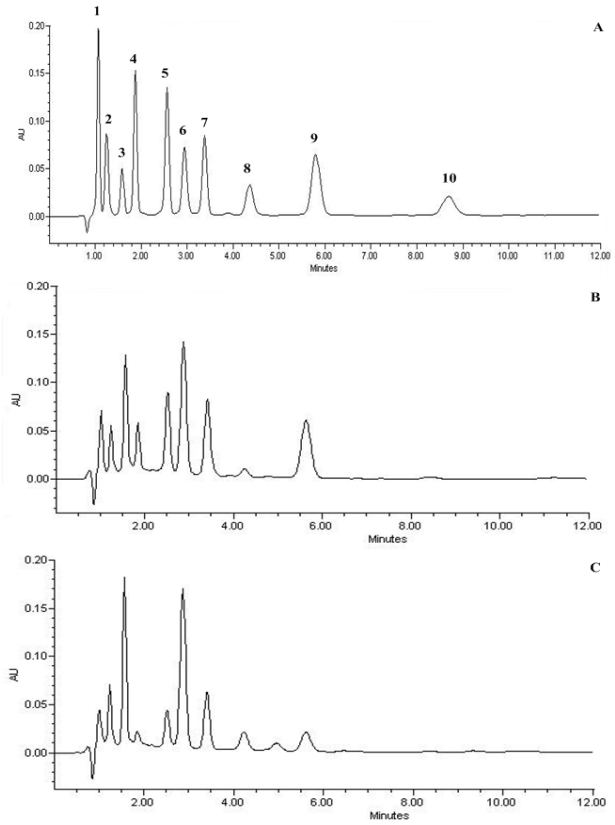

Figure 3. Typical chromatogram of standard compounds (A), fresh tea shoot from plucking step (B) and Oolong tea from drying step (C). Peak identification and approximate retention times in minutes in parentheses are as follows: 1. G (1.07); 2. GC (1.25); 3. EGC (1.59); 4. C (1.88); 5. EC (2.56); 6. EGCG (2.94); 7. CF (3.40); 8. GCG (4.36); 9. ECG (5.81); and 10. CG (8.71)

detected in the fresh tea shoots, and they were identified as G, GC, EGC, C, EC, EGCG, CF, GCG, and ECG, in order from left to right on the HPLC chromatograms, by comparing their retention times with those of the authentic standards. The CG was not detected in the fresh tea shoots. Quantification of G, GC, EGC, C, EC, EGCG, CF, GCG, and ECG was performed by comparing their peak area of the HPLC chromatograms with the standards. It was found that the fresh tea shoots contained 3.61±0.24 g/100 g DW of CF and 0.038±0.012 g/100 g DW of G. Among quantified individual catechins, EGCG was predominant (4.93±0.29 g/100 g DW), followed by EC (1.91±0.15 g/100 g DW), EGC (1.89±0.25 g/100 g DW), C (1.65±0.18 g/100 g DW), and trace amounts of GC (0.36±0.05 g/100 g DW) and GCG (0.25±0.04 g/100 g DW). By the summation of individual catechins, the amount of total catechins was 12.24±0.18 g/100 g DW. These results are in reasonable agreement with previous reports (Chen *et al*., 2010; Wu *et al*., 2012; Shitandi *et al*., 2013). However, there were some variations in the concentrations of individual phenolic compounds. This might be due to the fact that types and contents of chemical compounds are affected by many factors, including the sub-varieties and cultivar used, elements of the growing environment, and plucking conditions (Chen *et al.*, 2010; Lee *et al*., 2014).

Changes in caffeine content

During Oolong tea production, the concentration of CF remained constant (about 3.55-3.61 g/100 g DW) all along the process. This result was somewhat unexpected because many studies showed that the CF concentration was higher in fermented tea than non-fermented tea (Fernandez *et al.*, 2002; Cabrera *et al*., 2003; Lin *et al.*, 2003). However, there were reports that demonstrated a reduction of the CF level in partially-fermented tea and fermented teas (Zuo *et al.*, 2002; Del Rio *et al*., 2004). Thus, the reason why fermented tea was reported to contain more or less CF than non-fermented tea might be because of many factors such as species, season, age of the leaves (plucking position), climate, geographical difference horticultural conditions (soil, water, minerals, fertilizers, etc.), and processing methods.

It has been recently found that the concentration of CF decreased as the degree of fermentation increased from green tea (0%) to black tea (80%) when the starting material was the same. However, the CF concentration in green tea (0% fermentation) was not significantly different from partially-fermented tea (20% fermentation) (Kim *et al*., 2011). Shitandi *et al.* (2013) also reported that all the varieties of tea contain 3-6 g/100 g DW of caffeine which was unaffected by the different processing methods (Shitandi *et al*., 2013). These reports supported our results which found that the content of CF remained quite stable and did not affect by Oolong tea processing at the degree of fermentation about 10-20%.

Changes in gallic acid content

According to a study by Fernandez *et al.* (2002), the concentration of G in 45 tea samples, including fermented and non-fermented teas of different geographical areas ranged between 0.004 and 2.537 g/100 g DW and the lower percentages were obtained for the non-fermented teas (Fernandez *et al*., 2002). Our result showed that the concentrations of G in fresh tea leaves and Oolong teas were 0.038±0.012 g/100 g DW and 0.177±0.018 g/100 g DW, respectively. The one way analysis of variance demonstrated that the concentration of G significantly increased in pan-firing and drying steps. It has been reported that the fermentation process (indoor withering step) increased the liberation of G from CG as indicated by the remarkably high levels of this acid in pu-erh

and black teas (Zuo *et al*., 2002). In this study, the result obtained for G was not fully in agreement with any of the above report due to undetected CG. The increase of G may be due to the thermal degradation in pan-firing and drying steps. The galloyl groups of major esterified catechins (EGCG and/or ECG) may be cleaved during thermal process. This resulted in the increase of free G as can be seen in the decrease of EGCG and/or ECG.

Changes in total catechins

Originally, the amount of total catechins in fresh tea shoots was 12.24±0.18 g/100 g DW. During processing, the concentration of total catechins remained constant in the outdoor withering step. It significantly decreased in the indoor withering step (10.04±0.37 g/100 g DW) and slightly increased in the pan-firing step. After drying, the concentration of total catechins was 11.05±0.46 g/100 g DW, approximately 90% comparing to the original.

Changes in phenolic compounds

Tea catechins contain two aromatic rings and several hydroxyl groups which they can be categorized into two groups: free catechins and esterified catechins. The C, EC, GC and EGC are non-esterified whereas the esterified catechins are CG, ECG, GCG and EGCG. In addition, tea catechins can be classified, according to their stereochemical configurations, into "non-epicatechins or non epi-structure" (2, 3-*trans*, such as C, CG, GC, and GCG) and "epicatechins or epi-structure" (2, 3-*cis*, including EC, ECG, EGC, and EGCG). These *cis* compounds can convert to their epimers that are non-epicatechins, i.e., (–)-gallocatechin (GC), (–)-gallocatechin gallate (GCG), (–)-catechin gallate (CG), and (±)-catechin (C), respectively. This is called epimerization and the epimerization between pair catechins is reversible. The chemical structures of epicatechins and non-epicatechins only differ between 2R, 3R (2, 3-*cis*, epi-structure) and 2S, 3R (2, 3-*trans*, non epi-structure) (Wang and Helliwell, 2000; Xu *et al*., 2003)

The EC has an ortho-dihydroxyl group in the B-ring at carbons 3′ and 4′ and a hydroxyl group at carbon 3 on the C-ring. The C has the same structure as EC but it differs from EC in that it has a 2S, 3R (2, 3-*trans*, non epi-structure) while EC has a 2R, 3R (2, 3-*cis*, epi-structure) (Yilmaz, 2006). The concentrations of EC and C in fresh tea shoots were 1.91±0.15 g/100 g DW and 1.65±0.18 g/100 g DW, respectively. Their concentrations remained constant in the outdoor withering step and decreased steadily after the drying step to 0.83±0.12 g/100 g DW and 0.94±0.18 g/100 g DW, respectively. Significant decreases were found in the indoor withering and pan-firing steps for both EC and C. The reductions were approximately 81% and 43% for EC and 89% and 57% for C, respectively.

The EGC has the same structure as EC but it differs from EC in that it has a trihydroxyl group at carbons 3′, 4′, and 5′ on the B-ring. The GC has the same structure as EGC but it differs from EGC in that it has a 2S, 3R (2, 3-*trans*, non epi-structure) while EGC has a 2R, 3R (2, 3-*cis*, epi-structure) (Yilmaz, 2006). The concentration of EGC in the fresh tea shoots was 1.89±0.25 g/100 g DW. The EGC remained constant in the outdoor and significantly decreased in the indoor withering step. It considerably increased in the pan-firing step and then significantly increased in the drying step. The indoor withering step significantly affected the decreasing in EGC content by approximately 81% comparing to the original. This may be because enzymatic oxidation during a partial fermentation. Interestingly, two thermal processes (pan-firing and drying steps) remarkably increased the EGC content. This result was somewhat unexpected. The increase in EGC during thermal processes is probably due to the thermal degradation of EGCG. The EGCG might degrade, resulting in the formation of EGC and the liberation of free G. The concentration of GC, a non epi-structure of EGC, was found in trace amounts (0.36±0.05 g/100 g DW) in fresh tea shoots. The change of GC during processing could be also observed as its epi-structure, EGC. Many researchers have reported that tea catechins can be converted to their epimers during tea production, brewing and storage. Epimerization can occur at high temperature (Wang and Helliwell 2000). It has been recognized that catechins undergo epimerization at the C2-position in hot aqueous solution. This epimerization can change the epistructured catechin to non epi-structured catechin and vice versa. (Wang and Helliwell, 2000; Chen *et al.*, 2001; Ito *et al.,* 2003; Friedman *et al.*, 2009; Sharma and Zhou, 2011; Ananingsih *et al*., 2013). As found in this study that the quantity of GC increased, a possible reason responsible for the increase in GC could be due to the epimerization from EGC to GC in thermal processes.

The ECG has the same structure as EC but ECG differs from EC in its gallate moiety esterified at carbon 3 of the C-ring. The CG has the same structure as ECG but it differs from EGC in that it has a 2S, 3R (2, 3-*trans*, non epi-structure) while ECG has a 2R, 3R (2, 3-*cis*, epi-structure) (Yilmaz, 2006). The concentration of ECG, the second most esterified catechins, in fresh tea shoots was 1.15±0.09 g/100 g DW and Oolong teas were 0.70±0.05 g/100 g DW.

The one way analysis of variance showed that ECG was first slightly decreased in the indoor withering (1.01±0.30 g/100 g DW), and then slightly increased after the pan-firing step (1.18±0.46 g/100 g DW),. The decrease was significantly observed in rolling and drying steps. It indicated that indoor withering, rolling and drying steps significantly affected the decreasing in ECG content by approximately 88%, 71 and 60%, respectively, comparing with the original. The reason why the concentration of ECG was fluctuated could be due to many factors and reactions occurred during processing. During indoor withering, the ECG might undergo partial fermentation, resulting in enzymatic oxidation occuring with ECG, and forming dimers or highly complexed groups of compounds. During thermal processes, the ECG might degrade, resulting in the liberation of free G. In addition, it might be formed from the thermal degradation of the EGCG. In this study, the CG was not detected in fresh tea shoots and the increased quantity of CG by epimerizing from ECG could not be seen, indicating that the epimerization might not cause the decrease in ECG. We may, therefore, reasonably conclude that the alterations of ECG in tea leaves are quite complicated during Oolong tea processing but the changes do not come from the epimerzation.

The EGCG has the same structure as EGC but EGCG differs from EGC in its gallate moiety esterified at carbon 3 of the C-ring. The GCG has the same structure as EGCG but it differs from EGCG in that it has a 2S, 3R (2, 3-*trans*, non epi-structure) while EGCG has a 2R, 3R (2, 3-*cis*, epi-structure) (Yilmaz, 2006). The EGCG was found to be the most abundant esterified catechins in fresh tea shoots (4.93±0.29 g/100 g DW). During processing, the concentration of EGCG significantly decreased in the indoor withering step and remained quite constant until the rolling step. Its concentration significantly decreased again in the drying step. It was found that the indoor withering and the drying steps significantly affected the decreasing in EGCG content by approximately 80% and 21%, respectively, comparing to the original. It can be stated that Oolong tea processing strongly affected the stability of EGCG. It might be that EGCG is very susceptible to the oxidation under humidity in the indoor withering step. A possible reason responsible for the loss of EGCG during the indoor withering could be due to the enzymatic oxidation, forming a dimeric catechins and/or complexed phenolic compounds. The main reasons for the loss of EGCG during thermal processing might be due to epimerization and/or degradation under extremely high temperature in the drying step. This is in agreement with the previously reported (Li *et al.*, 2011) theoretical understanding of the chemical features of EGCG. EGCG is a polyphenolic compound containing an ester group. Under such conditions, EGCG can oxidize into dimers and/or degrade into EGC and/or epimerzation into GCG (Wang and Helliwell, 2000; Ito *et al*., 2003; Friedman *et al*., 2009).

The concentration of GCG in the fresh tea shoots was found in trace amounts, (0.25±0.04 g/100 g DW). The GCG was significantly decreased in the indoor withering and then tremendously increased in the pan-firing step. It significantly increased again in the drying step. After drying steps, the concentrations of GCG increased to 0.94±0.07 g/100 g DW. These changes were the same as its epi-structure, EGCG.

Impact of process unit operations on the changes of catechins

After young tea shoots were plucked by hand, they were withered in direct sunlight (outdoor withering). The concentrations of phenolic compounds of tea leaves in the outdoor withering step significantly remained constant, comparing to the young tea shoots. This indicated that the outdoor withering step did not affect the level of phenolic compounds. By observation, this step was performed for only moisture removal and emission of grassy odor in the fresh tea shoots.

Among the process unit operations, indoor withering, pan-firing and drying steps had significant impact on catechin concentrations. Indoor withering was a step that allowed the leaves to undergo a partial fermentation. The temperature and relative humidity were controlled at 20–25°C and 75–85%, respectively. During indoor withering step, the tea shoots were rotated, as a result the fermentation occured. During the fermentation of tea leaves, the monomeric catechins were subjected to the action of polyphenol oxidase. This enzyme caused oxidation of catechins to quinones which further underwent polymerization to bisflavans and to more complex structures of theaflavins, thearubigens and higher molecular mass compounds (Lin *et al.*, 1998). In this study, the degree of tea fermentation was controlled at approximately 10-20%. The results showed that the level of total catechins, EC, C, EGC, GC, ECG, EGCG and GCG decreased during partial fermentation. A possible reason responsible for the significant decrease in catechins could be due to the enzymatic oxidation, forming a dimeric catechins and/or complexed phenolic compounds.

The chemistry of withering has been studied by many scientists. During withering, about 15% of the chlorophylls were degraded to chlorophyllides

Table 1. Contents of moisture, total polyphenols, caffeine, gallic acid, total catechins and individual catechins of teas in each processing step

Parameter	Processing step					
	Plucking	Outdoor withering	Indoor withering	Pan-firing	Rolling	Drying
Moisture	76.29±3.59^{a}	71.88±1.94^{b}	68.16±3.45^{c}	23.70±2.82^{d}	11.56±2.73^{e}	2.07 ±0.20^{f}
Total polyphenols^{ns}	15.17±1.22	15.08±0.73	14.20±2.40	14.14±1.72	14.34±1.79	14.27±1.03
Caffeine^{ns}	3.61±0.24	3.59±0.23	3.55±0.37	3.57±0.33	3.61±0.36	3.55±0.33
Gallic acid	0.038±0.012^{c}	0.035±0.152^{c}	0.040±0.009^{c}	0.072±0.012^{b}	0.078±0.017^{b}	0.177±0.018^{a}
Total catechins	12.24±0.18^{a}	12.13±0.44^{a}	10.04±0.37^{c}	11.15±1.16^{b}	10.59±0.69^{bc}	11.05±0.46^{c}
EC	1.91±0.15^{a}	1.86±0.19^{a}	1.55±0.27^{b}	1.01±0.34^{c}	0.91±0.21^{c}	0.83±0.12^{c}
C	1.65±0.18^{a}	1.64±0.17^{a}	1.47±0.28^{ab}	1.22±0.35^{bc}	1.19±0.36^{bc}	0.94±0.18^{c}
EGC	1.89±0.25^{c}	1.86±0.24^{c}	1.53±0.28^{c}	2.60±0.29^{b}	2.63±0.23^{b}	3.17±0.24^{a}
GC	0.36±0.05^{c}	0.36±0.08^{c}	0.32±0.05^{c}	0.67±0.12^{b}	0.63±0.12^{b}	1.47±0.12^{a}
ECG	1.15±0.09^{a}	1.16±0.13^{a}	1.01±0.30^{ab}	1.18±0.46^{a}	0.82±0.04^{bc}	0.70±0.05^{c}
CG	nd	nd	nd	nd	nd	nd
EGCG	4.93±0.29^{a}	4.90±0.34^{a}	3.93±0.58^{b}	3.71±0.30^{b}	3.68±0.73^{b}	3.00±0.14^{c}
GCG	0.25±0.04^{c}	0.25±0.04^{c}	0.18±0.04^{d}	0.69±0.07^{b}	0.68±0.06^{b}	0.94±0.07^{a}

Values are expressed as means ± SD (n=3).
Different letters in the same row indicate significant difference at $p < 0.05$.
ns = not significant. nd = not detected

(Tomlins and Mashingaidze, 1997). Carotenoid degradation was found to yield large quantities of desirable aroma volatiles, giving a high flavor index (Ravichandran, 2002). Levels of catechins decreased with increased withering time through the enhanced activity of the enzyme polyphenol oxidase. It was reported that some amino acids increased the tea aroma and the influence of withering on the amino acid level varied with the duration, moisture and ambient temperature (Tomlins and Mashingaidze, 1997). Lipid metabolism was important for the formation of aroma compounds (Mahanta *et al.*, 1993). During withering, lipid degradation occured and the unsaturated fatty acid level decreased through oxidative cleavage to form aroma compounds. It was reported that the majority of changes in lipids occurred during withering rather than the other manufacturing stages (Ravichandran and Parthiban, 2000).

The pan-firing step involved placing leaves directly on a dry pan exposed to a high heat source of a pan-firing machine. The temperature for pan-firing was around 250-300˚C. Following pan-firing, leaves were rolled in order to disrupt their cell walls, resulting in releasing of leaf moisture, and then shaped into a ball-shape. Finally, the shaped leaves were dried by drying machine. As the leaves were heated, rolled and dried during processing, the total catechin content decreased as phenolic compounds underwent oxidation, hydrolysis, polymerization and transformation. Caffeine, on the other hand, was less sensitive to heat and did not undergo considerable reduction during processing.

As found that pan-firing and drying remarkably increased the level of EGC, GC and GCG. It can be seen from Table 1 that the amount of EGC, GC and GCG in the Oolong tea exceeded 100%, comparing to the fresh tea shoots. This clearly indicates that some other catechins or polyphenols were converted into EGC, GC and GCG during the pan-firing and drying steps. The increase in EGC during thermal processes is probably due to the thermal degradation of EGCG, resulting in the formation of EGC and the liberation of free G (as can be clearly seen that EGCG decreased while G increased). Degradation of EGCG was evident as there was a declining trend in EGCG in thermal processes. The epimerization of EGC and EGCG may cause the increase in GC and GCG because epimerization can occur at high temperature (Wang and Helliwell, 2000). This epimerization can change the epi-structured catechin to non epi-structured catechin. This fact can be supported by a study conducted by Hou *et al.* (2005), where it was found that GCG formed from EGCG further got converted into other products or radicals, and thus could not even be detected (Hou *et al.*, 2005). Although epimerization could change the epi-structured catechin to non epi-structured catechin and vice versa, the concentration of GCG increased while the concentration of EGCG decreased. This may be due to the content of the EGCG being higher than the GCG, the degree of conversion for EGCG was greater than that of GCG. Consequently, the content of EGCG was reduced towards the equilibrium of EGCG and GCG. As a result, epimerization of the catechins, especially EGCG, is thought to be one of the most important reactions in the manufacture of Oolong tea (Ito *et al.*, 2003; Friedman *et al.*, 2009; Sharma and Zhou, 2011; Ananingsih *et al.*, 2013). In addition, other possible reasons responsible for the changes of catechins could be due to available oxygen and metal ions in the processing. Sang *et al.*

(2005) reported that higher oxygen levels increased catechin oxidation (Sang *et al.*, 2005). Catechins could react with metal ions to form metal complexes. Kumamoto *et al.* (2001) reported that metal ions preferentially bound to the gallate group of ECG and EGCG (Kumamoto *et al*., 2001).

Conclusion

As a result of light Oolong tea manufacture, the contents of total polyphenols and CF significantly remained constant while the content of total catechins slightly decreased. Among the process unit operations, indoor withering, pan-firing and drying steps had impact on catechin concentrations. The changes of individual catechins were likely due to enzymatic oxidation, thermal degradation and epimerization. The tea market has grown extensively due to an increased awareness of the potential health benefits from bioactive polyphenolics present in teas. Thus, results obtained from the present study provide practical information to the tea industry concerning phenolic compounds.

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References

AOAC. 2002. Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals. Gaithersburg, Md.: AOAC Int. Downloaded from: *http://swww.aoac.org/dietsupp6/Dietary-Supplement-web-site/slv_guidelines.pdf*. Accessed Nov 30, 2011.

Ananingsih, V.K., Sharma, A. and Zhou, W. 2013. Green tea catechins during food processing and storage: A review on stability and detection. Food Research International 50: 469-479.

Bazinet, L., Araya-Farias, M., Doyen, A., Trudel, D. and Têtu, B. 2010. Effect of process unit operations and long-term storage on catechin contents in EGCG-enriched tea drink. Food Research International 43: 1692-1701.

Bruno, R.S., Bomser, J.A. and Ferruzzi, M.G. 2014. Chapter 4 - Antioxidant capacity of green tea *(Camellia sinensis)*. In: Preedy, V. (Ed.), Processing and impact on antioxidants in beverages. Academic Press, San Diego, pp. 33-39.

Cabrera, C., Gimenez, R. and Lopez, M.C. 2003. Determination of tea components with antioxidant activity. Journal of Agricultural and Food Chemistry 51: 4427-4435.

Chen, L., Chen, J., Guo, Y., Li, J., Yang, Y., Xu, L. and Fu, F. 2014. Study on the simultaneous determination of seven benzoylurea pesticides in Oolong tea and their leaching characteristics during infusing process by HPLC–MS/MS. Food Chemistry 143: 405-410.

Chen, Y.-J., Kuo, P.-C., Yang, M.-L., Li, F.-Y. and Tzen, J.T.C. 2013. Effects of baking and aging on the changes of phenolic and volatile compounds in the preparation of old Tieguanyin Oolong teas. Food Research International 53: 732-743.

Chen, Y., Jiang, Y., Duan, J., Shi, J., Xue, S. and Kakuda, Y. 2010. Variation in catechin contents in relation to quality of 'Huang Zhi Xiang' Oolong tea *(Camellia sinensis)* at various growing altitudes and seasons. Food Chemistry 119: 648-652.

Chen, Z., Zhu, Q.Y., Tsang, D. and Huang, Y. 2001. Degradation of green tea catechins in tea drinks. Journal of Agricultural and Food Chemistry 49: 477-482.

Del Rio, D., Stewart, A.J., Mullen, W., Burns, J., Lean, M.E., Brighenti, F. and Crozier, A. 2004. HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea. Journal of Agricultural and Food Chemistry 52: 2807-2815.

Dutta, P.P. and Baruah, D.C. 2014. Drying modelling and experimentation of assam black tea *(Camellia sinensis)* with producer gas as a fuel. Applied Thermal Engineering 63: 495-502.

Fernandez, P.L., Pablos, F., Martin, M.J. and Gonzalez, A.G. 2002. Study of catechin and xanthine tea profiles as geographical tracers. Journal of Agricultural and Food Chemistry 50: 1833-1839.

Friedman, M., Levin, C.E., Lee, S.U. and Kozukue, N. 2009. Stability of green tea catechins in commercial tea leaves during storage for 6 months. Journal of Food Science 74: 1750-3841.

Grant, P. and Dworakowska, D. 2013. Chapter 55 - Tea and diabetes: The laboratory and the real world. In: Preedy, V.R. (Ed.), Tea in health and disease prevention. Academic Press, pp. 661-674.

He, R.-R., Kurihara, H. and Preedy, V.R. 2013. Chapter 42 - Oolong tea and weight loss. In: Preedy, V.R. (Ed.), Tea in health and disease prevention. Academic Press, pp. 509-519.

Horanni, R. and Engelhardt, U.H. 2013. Determination of amino acids in white, green, black, Oolong, pu-erh teas and tea products. Journal of Food Composition and Analysis 31: 94-100.

Hou, Z., Sang, S., You, H., Lee, M.J., Hong, J., Chin, K.V. and Yang, C.S. 2005. Mechanism of action of (-)-epigallocatechin-3-gallate: auto-oxidation-dependent inactivation of epidermal growth factor receptor and direct effects on growth inhibition in human esophageal cancer KYSE 150 cells. Cancer Research 65: 8049-8056.

ISO 14502–1. 2005. Tea; Methods for determination of substances characteristic of green and black tea. Part 1, Content of total polyphenols in tea: colorimetric method using Folin–Ciocalteu reagent.

ISO 14502–2. 2005. Tea; Methods for determination of substances characteristic of green and black tea. Part 2. Contents of catechins in green tea: method using

high performance liquid chromatography.

Ito, R., Yamamoto, A., Kodama, S., Kato, K., Yoshimura, Y., Matsunaga, A. and Nakazawa, H. 2003. A study on the change of enantiomeric purity of catechins in green tea infusion. Food Chemistry 83: 563-568.

Kapiszewska, M., Merklinger-Gruchala, A. and Jasienska, G. 2013. Chapter 46 - The modulating influence of tea polyphenols on estrogen metabolism in asian and caucasian populations: Breast Cancer Prevention. In: Preedy, V.R. (Ed.), Tea in health and disease prevention. Academic Press, pp. 551-562.

Kaur, L., Jayasekera, S. and Moughan, P.J. 2014. Chapter 13 - Antioxidant quality of tea *(Camellia sinensis)* as affected by environmental factors. In: Preedy, V. (Ed.), Processing and impact on antioxidants in beverages. Academic Press, San Diego, pp. 121-129.

Kausar, T., Akram, K. and Kwon, J.-H. 2013. Comparative effects of irradiation, fumigation, and storage on the free amino acids and sugar contents of green, black and Oolong teas. Radiation Physics and Chemistry 86: 96-101.

Kim, Y., Goodner, K.L., Park, J.-D., Choi, J. and Talcott, S.T. 2011. Changes in antioxidant phytochemicals and volatile composition of *Camellia sinensis* by oxidation during tea fermentation. Food Chemistry 129: 1331-1342.

Kosińska, A. and Andlauer, W. 2014. Chapter 12 - Antioxidant capacity of tea: effect of processing and storage. In: Preedy, V. (Ed.), Processing and impact on antioxidants in beverages. Academic Press, San Diego, pp. 109-120.

Kumamoto, M., Sonda, T., Nagayama, K. and Tabata, M. 2001. Effects of pH and metal ions on antioxidative activities of catechins. Bioscience, Biotechnology, and Biochemistry 65: 126-132.

Labbé, D., Têtu, B., Trudel, D. and Bazinet, L. 2008. Catechin stability of EGC- and EGCG-enriched tea drinks produced by a two-step extraction procedure. Food Chemistry 111: 139-143.

Lee, M.-S., Hwang, Y.-S., Lee, J. and Choung, M.-G. 2014. The characterization of caffeine and nine individual catechins in the leaves of green tea (*Camellia sinensis* L.) by near-infrared reflectance spectroscopy. Food Chemistry 158: 351-357.

Li, N., Taylor, L.S., Ferruzzi, M.G. and Mauer, L.J. 2012. Kinetic study of catechin stability: effects of pH, concentration, and temperature. Journal of Agricultural and Food Chemistry 60: 12531-12539.

Li, N., Taylor, L.S., Mauer and L.J. 2011. Degradation kinetics of catechins in green tea powder: effects of temperature and relative humidity. Journal of Agricultural and Food Chemistry 59: 6082-6090.

Li, N., Taylor, L.S. and Mauer, L.J. 2014. The physical and chemical stability of amorphous (−)-epi-gallocatechin gallate: Effects of water vapor sorption and storage temperature. Food Research International 58: 112-123.

Lin, J.-K., Lin, C.-L., Liang, Y.-C., Lin-Shiau, S.-Y. and Juan, I.M. 1998. Survey of catechins, gallic acid, and methylxanthines in green, Oolong, pu-erh, and black Teas. Journal of Agricultural and Food Chemistry 46: 3635-3642.

Lin, Y.S., Tsai, Y.J., Tsay, J.S. and Lin, J.K. 2003. Factors affecting the levels of tea polyphenols and caffeine in tea leaves. Journal of Agricultural and Food Chemistry 51: 1864-1873.

Mahanta, P.K., Tamuli, P. and Bhuyan, L.P. 1993. Changes of fatty acid contents, lipoxygenase activities, and volatiles during black tea manufacture. Journal of Agricultural and Food Chemistry 41: 1677-1683.

Maria John, K.M., Thiruvengadam, M., Enkhtaivan, G. and Kim, D.H. 2014. Variation in major phenolic compounds and quality potential of CTC black tea elicited by *Saccharomyces cercevisiae* and its correlation with antioxidant potential. Industrial Crops and Products 55: 289-294.

Monsanto, M., Hooshyar, N., Meuldijk, J. and Zondervan, E. 2014. Modeling and optimization of green tea precipitation for the recovery of catechins. Separation and Purification Technology 129: 129-136.

Namal Senanayake, S.P.J. 2013. Green tea extract: Chemistry, antioxidant properties and food applications – A review. Journal of Functional Foods 5: 1529-1541.

Owuor, P.O., Obanda, M., Nyirenda, H.E. and Mandala, W.L. 2008. Influence of region of production on clonal black tea chemical characteristics. Food Chemistry 108: 263-271.

Pan, M.-H., Lai, C.-S., Wang, H., Lo, C.-Y., Ho, C.-T. and Li, S. 2013. Black tea in chemo-prevention of cancer and other human diseases. Food Science and Human Wellness 2: 12-21.

Peterson, J., Dwyer, J., Bhagwat, S., Haytowitz, D., Holden, J., Eldridge, A.L., Beecher, G. and Aladesanmi, J. 2005. Major flavonoids in dry tea. Journal of Food Composition and Analysis 18: 487-501.

Ravichandran, R. 2002. Carotenoid composition, distribution and degradation to flavour volatiles during black tea manufacture and the effect of carotenoid supplementation on tea quality and aroma. Food Chemistry 78: 23-28.

Ravichandran, R. and Parthiban, R. 2000. Lipid occurrence, distribution and degradation to flavour volatiles during tea processing. Food Chemistry 68: 7-13.

Riegsecker, S., Wiczynski, D., Kaplan, M.J. and Ahmed, S. 2013. Potential benefits of green tea polyphenol EGCG in the prevention and treatment of vascular inflammation in rheumatoid arthritis. Life Sciences 93: 307-312.

Sang, S., Lambert, J.D., Ho, C.-T. and Yang, C.S. 2011. The chemistry and biotransformation of tea constituents. Pharmacological Research 64: 87-99.

Sang, S., Lee, M.J., Hou, Z., Ho, C.T. and Yang, C.S. 2005. Stability of tea polyphenol (-)-epigallocatechin-3-gallate and formation of dimers and epimers under common experimental conditions. Journal of Agricultural and Food Chemistry 53: 9478-9484.

Senthil Kumar, R.S., Murugesan, S., Kottur, G. and Gyamfi, D. 2013. Chapter 4 - Black Tea: The plants, processing/manufacturing and production. In: Preedy,

V.R. (Ed.), Tea in health and disease prevention. Academic Press, pp. 41-57.
Sharma, A. and Zhou, W. 2011. A stability study of green tea catechins during the biscuit making process. Food Chemistry 126: 568-573.
Sharma, R. 2014. Chapter 59 - Polyphenols in health and disease: Practice and mechanisms of benefits. In: Watson, R.R., Preedy, V.R., Zibadi, S. (Eds.), Polyphenols in human Health and Disease. Academic Press, San Diego, pp. 757-778.
Shitandi, A.A., Muigai Ngure, F. and Mahungu, S.M. 2013. Chapter 16 - Tea processing and its impact on catechins, theaflavin and thearubigin formation. In: Preedy, V.R. (Ed.), Tea in health and disease prevention. Academic Press, pp. 193-205.
Singh, B.N., Shankar, S. and Srivastava, R.K. 2011. Green tea catechin, epigallocatechin-3-gallate (EGCG): Mechanisms, perspectives and clinical applications. Biochemical Pharmacology 82: 1807-1821.
Stodt, U. and Engelhardt, U.H. 2013. Progress in the analysis of selected tea constituents over the past 20 years. Food Research International 53: 636-648.
Tomlins, K.I. and Mashingaidze, A. 1997. Influence of withering, including leaf handling, on the manufacturing and quality of black teas — a review. Food Chemistry 60: 573-580.
Tontul, I., Torun, M., Dincer, C., Sahin-Nadeem, H., Topuz, A., Turna, T. and Ozdemir, F. 2013. Comparative study on volatile compounds in Turkish green tea powder: Impact of tea clone, shading level and shooting period. Food Research International 53: 744-750.
Wachira, F.N., Karori, S., Kerio, L.C. and Wanyoko, J.K. 2013. Chapter 8 - Cultivar type and antioxidant potency of tea product. In: Preedy, V.R. (Ed.), Tea in health and disease prevention. Academic Press, pp. 91-102.
Wang, H., Provan, G.J. and Helliwell, K. 2000. Tea flavonoids: their functions, utilisation and analysis. Trends in Food Science and Technology 11: 152-160.
Wu, C., Xu, H., Héritier, J. and Andlauer, W. 2012. Determination of catechins and flavonol glycosides in Chinese tea varieties. Food Chemistry 132: 144-149.
Xu, J.Z., Leung, L.K., Huang, Y. and Chen, Z.-Y. 2003. Epimerisation of tea polyphenols in tea drinks. Journal of the Science of Food and Agriculture 83: 1617-1621.
Yilmaz, Y. 2006. Novel uses of catechins in foods. Trends in Food Science and Technology 17: 64-71.
Zuo, Y., Chen, H., and Deng, Y. 2002. Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. Talanta 57: 307-316.