CHAPTER

12

Antioxidant Capacity of Tea: Effect of Processing and Storage

Agnieszka Kosińska[*,†], *Wilfried Andlauer*[†]

[*]Division of Food Sciences, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland, [†]Institute of Life Technologies, University of Applied Sciences Valais, Sion, Switzerland

CHAPTER POINTS

- Processing of tea leaves results in profound differences in phenolic compound composition and antioxidant capacity of different types of tea.
- The more processed the tea, the lower its antioxidant capacity.
- Antioxidant activity of ready-to-drink tea beverages is much lower in comparison to tea-cup-prepared tea.
- Extended storage, being necessary to develop desirable taste and aroma of pu-erh and old oolong tea, can lead to loss of quality in white, yellow, green, and black tea.
- The lower the oxygen permeability of packaging material of ready-to-drink tea, the longer its storage stability.

INTRODUCTION

Tea plant is an evergreen shrub or small tree of the *Camelia* genus, native to China, with dark green shiny leaves and white flowers. *Camelia sinensis* var. *sinensis* and *Camelia sinensis* var. *assamica* are the two varieties most commonly used for the production of different types of tea. Freshly prepared infusion of dried leaves is a beverage consumed on a daily basis worldwide. Tea beverage is prepared at home by pouring boiling water over processed tea leaves. Tea is valued for its taste, aroma, health benefits, and cultural practices.

Fresh tea leaves are plucked manually or using a plucking machine. Most preferably, just two fresh leaves and the buds should be used, since it influences the quality and therefore the price of the tea. The younger the leaves used for the production are, the more expensive is the final product. Processing of tea leaves allows the production of various types of tea: white, yellow, oolong, green, black, and pu-erh, depending on the extent of the oxidation/fermentation process. Generally, leaves of the *sinensis* variety are used mainly for the production of green tea, while black and pu-erh tea are produced from *assamica* (Zhang *et al.*, 2012). It is estimated that about 78% of world production of tea accounts for black tea, 20% for green tea, and only 2% for other tea types. Black tea is consumed all over the world, whereas green and oolong tea, previously peculiar to Asia are also now gaining popularity in Europe and North America. White and pu-erh tea is mainly consumed in Asia. The increased popularity of green tea in Western countries results from a number of studies linking tea consumption with beneficial health effects. These beneficial effects are associated mainly with antioxidant properties of tea polyphenols. Moreover, antimutagenic, anticarcinogenic, hypocholesterolomic, antibacterial, and antiallergenic effects of tea have been reported (Cooper, 2011).

The composition of bioactive compounds, and therefore antioxidant capacity of tea, might be influenced by several parameters associated with growth conditions: genetic strain, climatic conditions, soil profile, growth altitude, horticultural practices, or plucking season. Interesting results on the effects of shade growth on tea phytochemical composition and antioxidant capacity were presented by Ku *et al.* (2010). Tencha, shade grown tea, showed generally lower antioxidant capacity (and epicatechin, epigallocatechin, and galloylquinic acid content) but higher content of amino acids than green tea.

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The other factors that may profoundly influence profile of bioactives in tea are processing, storage, and home preparation practices. They are considered in this review.

ANTIOXIDANT COMPOUNDS OF TEA

Tea is well known for its antioxidant effect. Several compounds present in tea are responsible for this activity.

Flavan-3-ols

Green tea is one of the most abundant food sources of catechins, members of flavan-3-ols, a subclass of flavonoids. Flavan-3-ols have two chiral centers C2 and C3, which results in four isomers for each level of B ring hydroxylation. (+)-Catechins and (−)-epicatechin are widespread in nature whereas (−)-catechins and (+)-epicatechin are rare. Catechins are characterized by di-or tri-hydroxyl group substitution of the B ring and meta-5,7-dihydoxysubstitution of the A ring. The most abundant catechins in tea are (−)-epigallocatechin gallate (EGCG), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (+)-catechin (C), and (−)-gallocatechin gallate (GCG). Tea catechins can undergo reversible epimerization, mainly as an effect of high-temperature treatment. It was reported that radical-scavenging ability of EGCG was higher than that of other catechins due to the presence of a gallate moiety in the C ring (Xu *et al.*, 2004). The following order of antioxidant activity of tea catechins emerged from a number of studies: epigallocatechin ~ epigallocatechin gallate >> epicatechin gallate = epicatechin > catechins (Łuczaj and Skrzydlewska, 2005). The number of hydroxyl groups is the major factor contributing to antioxidant potential (Rice-Evans *et al.*, 1997).

Phenolic Acids

Gallic acid and its quinic acid ester, theogallin (Figure 12.1), are the most abundant simple polyphenols present in tea. During processing of black tea, the amount of gallic acid significantly increases due to oxidative degallation of phenolic esters during the fermentation. Recently, 15 different phenolic acid derivatives have been identified in black tea by Lin *et al.* (2008).

Flavonols and Flavones

Quercetin, myricetin, kaempferol and their mono-, di-, and tri-glycosides are the most abundant flavonols in tea. Recently, three flavonols, 19 *O*-glycosylated flavonols, 28 acylated glycosylated flavonols, and seven C-glycosylated flavonols have been identified in white, green, oolong, and black tea samples (Lin *et al.*, 2008).

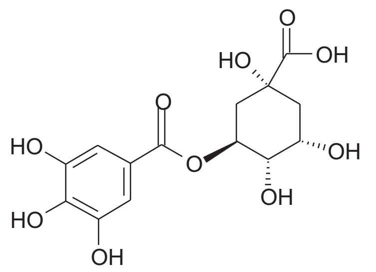

FIGURE 12.1 Chemical structure of theogallin. Theogallin and gallic acid are the most common simple phenolics in tea.

Theaflavins and Thearubigins

Theaflavins and thearubigins are characteristic products formed from catechins during enzymatic oxidation of tea during manufacturing. Theaflavins give an orange or orange–red color to tea and contribute to a mouthfeel sensation and an extent of cream formation. They are dimeric compounds that possess a benzotropolone skeleton that is formed from co-oxidation of selected pairs of catechins. The oxidation of the B ring of either (−)-epigallocatechin or (−)-epigallocatechin gallate is followed by loss of CO_2 and simultaneous fusion with the B ring of (−)-epicatechin or (−)-epicatechin gallate molecule (Figure 12.2). Four major theaflavins have been identified in black tea: theaflavin, theaflavin-3-monogallate, theaflavin-3′-monogallate, and theaflavin-3,3′-digallate. Additionally, their stereoisomers and derivatives can be present. Recently, the presence of theaflavin trigallate and tetragallate in black tea was reported (Chen *et al.*, 2012). The theaflavins can be further oxidized. They are probably also the precursors for the formation of polymeric thearubigins. However, the mechanism of reaction is not known hitherto. Thearubigins are red–brown or dark-brown pigments in black tea, their content accounts for up to 60% of dry weight of tea infusion. The structure of thearubigins is yet to be revealed. Presumably their molecular weight does not exceed 2100 Da (Haslam, 2003).

The antioxidant activity of theaflavins is comparable to that of catechins (Łuczaj and Skrzydlewska, 2005). Moreover, theaflavins were shown to be able to scavenge superoxide radicals more efficiently and 10-times faster than EGCG.

Theasinensins

Theasinensins are dimeric gallocatechins linked by C–C bonds between two B rings forming a biphenyl (Figure 12.3). They are present mainly in oolong tea. Theasinensin A is a product of oxidation of two EGCG molecules, B of EGCG and EGC, C of two EGC molecules, and F of EGCG and ECG. Additionally, theasinensins might be formed from gallocatechins in the gut during

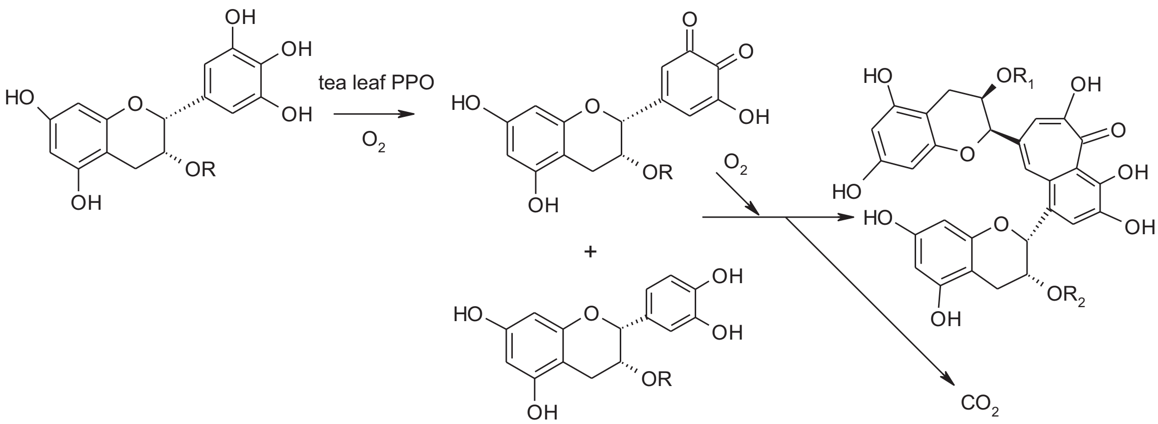

FIGURE 12.2 Mechanism of theaflavin formation. Theaflavins are formed as a result of co-oxidation of selected pairs of catechins catalyzed by polyphenol oxidase (PPO). R = H – EGC, R = galloyl group – EGCG, $R_1 = R_2 = H$ – theaflavin; R_1 = galloyl group, $R_2 = H$ and $R_1 = H$, R_2 = galloyl group – theaflavin gallate; $R_1 = R_2$ = galloyl group – theaflavin digallate.

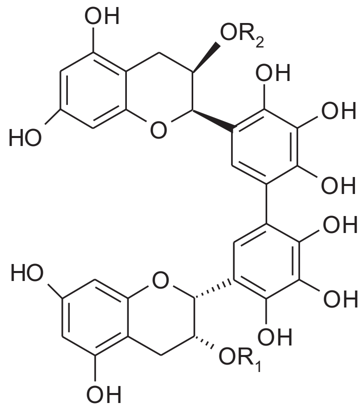

FIGURE 12.3 Chemical structure of theanisenisins: theanisenisin A: $R_1 = R_2$ = galloyl group; theanisenisin B: R_1 = galloyl group, $R_2 = H$ and $R_1 = H$, R_2 = galloyl group; theanisenisin C: $R_1 = R_2 = H$.

digestion of tea (Neilson *et al.*, 2010). It was reported that radical-scavenging activity of theasinensin A and D is comparable with that of EGCG on a molar basis (Yoshino *et al.*, 1999). In the case of thearubigins, their contribution to the antioxidant capacity of black tea is assumed.

Theabrownins

Theabrownins are pigment compounds formed during oxidation of tea leaves. They dissolve easily in water and not in organic solvents like ethyl acetate. They are the main bioactive components in pu-erh tea, and are characterized by high molecular weight and complex structure. It is known that theabrownins are formed by oxidation of polyphenols. However, the mechanism of their formation is not clear. It was reported that the cellulases and pectinases produced by microorganisms, together with polyphenol oxidase, play an important role in theabrownin formation (Wang *et al.*, 2011).

EFFECT OF PRODUCTION AND PROCESSING

Different tea types are obtained as result of differentiated processing, which substantially influence tea antioxidant composition.

Phenolic Compounds in Fresh Tea Leaves

Fresh tea leaves and unfermented tea types contain mainly flavan-3-ols, *O*-glycosylated flavonols, *C*-glycosylflavones, proanthocyanidins, phenolic acids, and their derivatives, whereas fermented tea contains also theaflavins and thearubigins. Recently, 24 phenolic compounds have been identified in tea leaves of different varieties by Wu *et al.* (2012). Leaves of eight tea varieties that are used for production of green, oolong, and black teas, were harvested, steamed to preserve oxidation, dried, and subsequently analyzed to evaluate the content of individual phenolic compounds. A unique phenolic compounds profile of each variety was reported. The contents of total catechins in the tea varieties for green tea manufacturing were all lower than the other two groups. EGCG was the major catechin in all tea varieties, accounting for about half of the total catechins. In the cited study, varieties for oolong tea manufacturing had the lowest content of total flavone and flavonol glycosides, whereas the green tea varieties had the highest content of these compounds.

Manufacturing Process

On the basis of the production process, tea can be divided into non-fermented (white, yellow, and green tea), semi-fermented or partly fermented (oolong tea), fermented (black tea), and post-fermented (pu-erh).

There are several steps in the production process that, selectively combined, determine which kind of tea will be obtained. The flow chart of production processes for different types of tea is depicted in Figures 12.4 and 12.5.

Withering

After the plucking, tea leaves undergo wilting or withering. Usually it takes place in the sun or on a rack in a heated room. Withering aims to remove moisture and soften the leaves to prepare them for further rolling. It leads also to the development of aroma and partial oxidation due to a breakdown of cell walls caused by moisture loss. Duration of withering depends on type of tea: white tea leaves are withered for 4–5h, whereas green, oolong and black tea are withered for at least twice as long (Cooper, 2011).

Steaming/Pan-Firing

Deactivation of enzymes, which initiates leaves oxidation, is a crucial process in the production of green tea. It can be achieved by quick application of high temperature either with steam, typical for Japanese-style green tea, or by dry heating called pan-firing, which is the traditional Chinese method. The temperature of pan-firing can reach 230°C, which is much higher than steaming temperature of 100°C (Wang *et al.*, 2000). Therefore, the method chosen for this step crucially affects the characteristics of the tea product obtained.

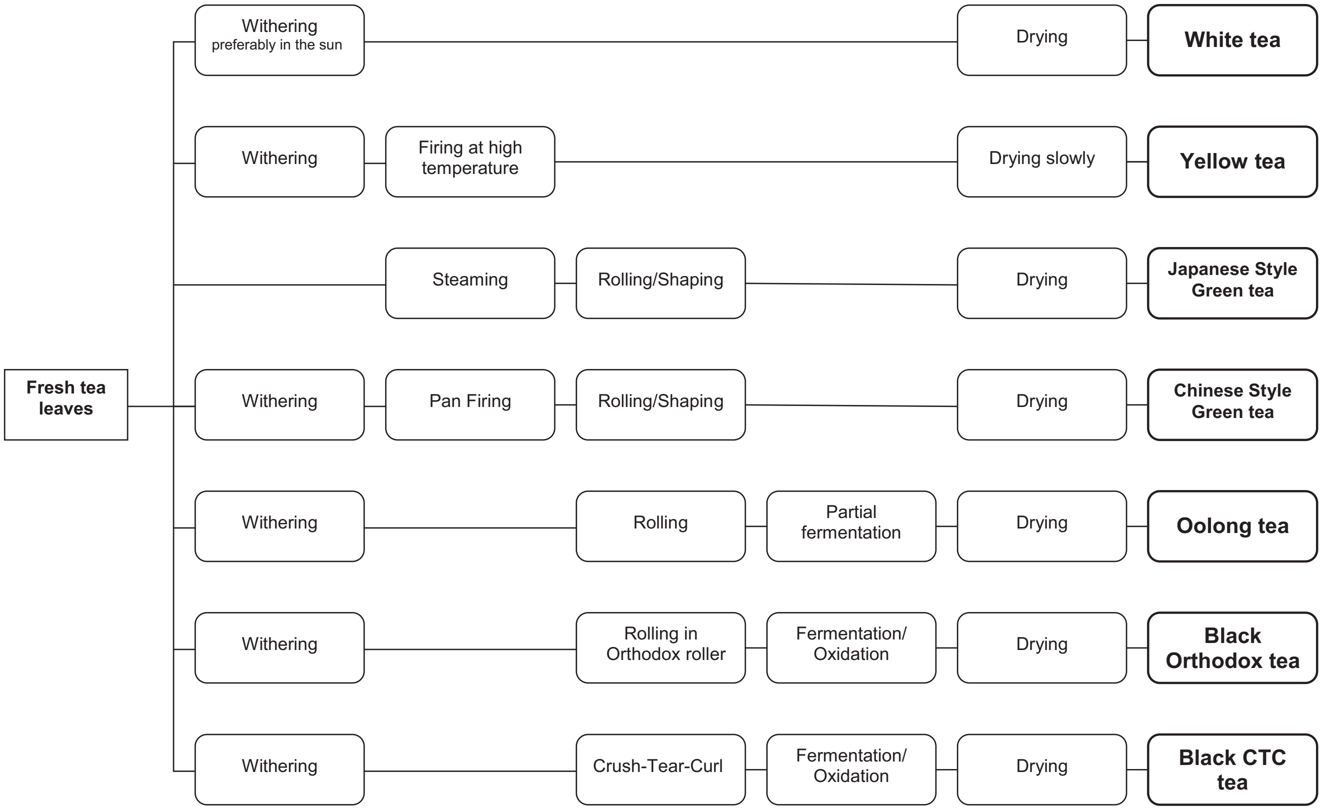

FIGURE 12.4 Flow chart of processing of different types of tea: white, yellow, green, oolong, black CTC, crush-tear-curl.

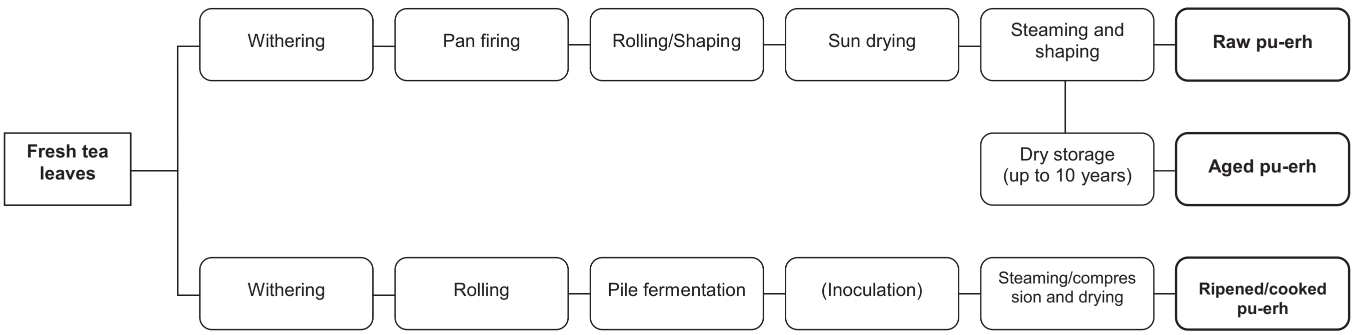

FIGURE 12.5 Flow chart of processing of different types of pu-erh tea.

Rolling

The rolling/twisting of tea leaves was originally done manually, whereas nowadays it is performed by machines. The aim of this process is to disrupt tea leaves in order to release tea oils and to enable the access of enzymes to polyphenols, which is essential for the oxidation process. There are two major types of processing: orthodox and cut-tear-curl (CTC). Orthodox rolling refers to hand processing or rolling with machines that imitate hand rolling. CTC machines enable more rapid and extensive leaf disruption, producing smaller particles and therefore greater surface areas for enzymatic oxidation. Tea obtained by this method is used mainly for commercial tea in the form of teabags.

Oxidation/Fermentation

Rolled leaves are placed on trays and left in a room with controlled temperature, humidity, and aeration for 2–4 h. The process is traditionally called fermentation; however, it is mainly enzymatic oxidation of polyphenols by polyphenol oxidases. The initial green color of leaves turns into light brown, and deep brown in the course of their oxidation, which indicates formation of oligomeric theaflavins and polymeric thearubigins.

Drying

The last step in tea manufacturing is drying of tea leaves to stop the fermentation and to reach the favorable moisture content to suppress microorganism growth. Thereafter, tea is sorted and packed.

Changes in Phenolic Composition Caused by Processing of Tea Leaves

The manufacturing process evokes profound changes in the phenolic compound profile, in the content of individual compounds as well as in the antioxidant capacity of final tea products. Therefore, depending on the type of tea, the final products contain highly distinct profiles of antioxidant compounds. The identification of 92 phenolic compounds in a total of 66 non-fermented and fermented teas collected from all around the world allowed the classification of all types of tea into five groups (Lin *et al.*, 2008). The first group, characterized by a high concentration of acylated flavonol glycosides, consisted of high-grade teas made of the younger buds and leaves harvested in the early leaf-growing stage. High content of catechins and glycosylated flavonols was typical for the group of common green teas. The profile of the teas belonging to third group (partially fermented oolong and pu-erh teas) was characterized by lower content of EGCG and presence of theaflavins, whereas in the teas of the fourth group (black) most of EGCG was oxidized to theaflavins. Only traces of EGCG were present in overfermented teas, i.e., pu-erh (fifth group).

A number of studies carried out have aimed to characterize of different types of commercially available teas in specific regions or around the world. An overview of these studies is presented in Table 12.1.

White Tea

White tea is made mainly from newly grown buds and young leaves with tiny, silvery hairs not exposed to sunlight to prevent chlorophyll production. Buds and leaves for white tea production are harvested only once a year in the early spring. Buds are plucked before they are open, then withered and air dried in the shade, under sunshine, or in a temperature-controlled room. White tea is the least processed type of tea. It is considered as a non-fermented type, however, a slight fermentation occurs since the processing lacks the step of enzyme deactivation. In line with this statement, the results presented by Zhang *et al.* (2011) revealed in white tea the presence of compounds characteristic for black tea mainly, and not found in any other tea type analyzed by the authors, i.e., aged pu-erh, ripened pu-erh, green, yellow, oolong (Table 12.1A). The compounds were identified as theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3′-digallate. Also the content of gallic acid in white tea is high and comparable to that of black and pu-erh teas. Another study showed that the total content of catechins in white tea was similar to that of green tea and higher than in green pu-erh tea (Zhao *et al.*, 2011). In all types of tea analyzed, EGCG was the predominant catechin, and its content was the highest in white tea, followed by green and green pu-erh. White tea was also the most abundant source of proanthocyanidins, phenolic acid derivatives and acylated glycosylated flavonols (Table 12.1B). Green tea showed significantly higher radical-scavenging activity compared to white tea (Unachukwu *et al.*, 2010). Total catechin content in both white tea and green tea was highly variable for different tea brands analyzed (Table 12.1C).

Yellow Tea

Yellow tea is the least produced and known type of tea. The production process involves mild pan-firing until 40% of dryness, and subsequently to 70% dryness. Still-hot tea leaves are packed in thick paper or cloth and stored in wooden or bamboo boxes for 7 days. The other possible way of obtaining yellow tea is to remove pan-fired tea leaves, cover them with cloth and allow resting for a few hours. The processing is repeated a few times until leaves are smothered and have gained appropriate taste and color. The final product is slightly oxidized and characterized by a milder taste. As can be seen in Table 12.1B, yellow tea has a phenolic composition like green tea,

TABLE 12.1 An Overview of the Studies Carried Out on the Phenolic Compounds Composition of Different Types of Tea

Compound	Green	Green/Aged Pu-erh	White	Yellow	Oolong	Black	Pu-erh
(A) Study characteristics 71 tea samples: 14 ripened pu-erh, 14 aged pu-erh, 17 green tea, 11 oolong tea, 7 black tea, 6 white tea, 2 yellow tea, water infusions were prepared; results were expressed as mg/g; average calculated for tea type (Zhang *et al.*, 2011)							
Gallic acid	1.21 ± 0.98	1.47 ± 1.27	4.62 ± 1.10	2.63	0.79 ± 0.69	3.91 ± 0.60	6.51 ± 2.98
Total catechins	66.38 ± 35.87	38.69 ± 16.51	70.73 ± 22.06	72.29	87.82 ± 35.13	7.79 ± 1.78	4.28 ± 1.01
(EGCG)	33.80 ± 19.20	9.43 ± 4.27	35.79 ± 12.38	35.58	30.81 ± 10.91	1.43 ± 0.43	0.08 ± 0.07
(B) Study characteristics 15 tea samples: 5 white, 5 green, and 5 green pu-erh, tea powder was extracted with 60% aqueous methanol, results expressed as mg/g; average calculated for tea type (Zhao *et al.*, 2011)							
Gallic acid	27.98 ± 9.08	46.56 ± 14.54	74.73 ± 31.81	—	—	—	—
Total catechins	213.74 ± 52.97	131.03 ± 58.91	200.94 ± 15.61	—	—	—	—
(EGCG)	144.22 ± 29.12	88.34 ± 37.54	169.77 ± 4.76	—	—	—	—
Total glycosylated flavonols	15.26 ± 3.98	11.75 ± 3.03	15.81 ± 3.67	—	—	—	—
Total acylated glycosylated flavonols	0.26 ± 0.11	0.36 ± 0.14	0.84 ± 0.71	—	—	—	—
Total proanthocyanidins	1.81 ± 0.95	0.87 ± 0.32	4.67 ± 2.72	—	—	—	—
(C) Study characteristics 26 tea samples: 8 types of white tea of two subtypes white peony and Yin Zhen silver needle; 18 samples of green tea representing 5 subtypes (dragonwell, gunpowder, jasmine pearl, sencha, and gykuro); results were expressed as mg/g; average calculated for tea type for the purpose of this review (Unachukwu *et al.*, 2010)							
Total catechins	109.71 ± 64.83	—	84.25 ± 117.89	—	—	—	—
(D) Study characteristics 8 tea samples: 4 green tea (Meifoo green, Shanghai green, Hangzhou Lung Ching and Jasmine tea), 2 oolong tea (Fujian and Jiangxi), 1 pu-erh, and 1 black tea; tea powder was extracted with 80% methanol; results were expressed as mg/g; average calculated for tea type for the purpose of this review (Zuo *et al.*, 2002)							
Gallic acid	1.02 ± 0.63	—	—	—	1.54 ± 0.18	2.06	5.53
Total catechins	110.29 ± 10.25	—	—	—	47.33 ± 8.73	15.31	12.78
EGCG	55.10 ± 5.02	—	—	—	25.20 ± 4.24	3.79	1.99
(E) Study characteristics Samples of Indonesian green and black tea. Water infusions were prepared by adding 18 ml of boiling water to 1 g of leaves and brewing for 3 min; the results were expressed as mg/l (Del Rio *et al.*, 2004)							
Gallic acid	6.0 ± 0.1	—	—	—	—	125 ± 7.5	—
Total catechins	4572	—	—	—	—	101	—

EGCG	1255 ± 63	—	—	—	—	19 ± 0.1	—
Total flavonols	778	—	—	—	—	570	—
Total theaflavins	nd	—	—	—	—	224	—
(F) Study characteristics 11 samples of green, oolong and black tea purchased in Hong Kong. The results were expressed as mg/g of dry leaves; average calculated for tea type for the purpose of this review (Chen *et al.*, 2001)							
Total catechins	111.2 ± 25.1	—	—	—	45.6 ± 3.9	3.8 ± 1.3	—
EGCG	68.74 ± 21.87	—	—	—	31.8 ± 3.5	2.0 ± 1.0	—
(G) Study characteristics 50 samples of black and green tea purchased on the German market, the results were expressed as mg/g; average calculated for tea type (Hilal and Engelhardt, 2007)							
Total catechins	15.1	—	—	—	—	14.2	—
Total theaflavins	nd	—	—	—	—	0.94	—
(H) Study characteristics 7 samples of black tea purchased on local market: 3 Chinese black tea (Keemun, Yingde and Yunnan black tea), 3 India black tea (Dajeeling, Assam and Nierjeeli) and 1 Sri Lanka black tea (Uva); were expressed as mg/g of dry leaves; average calculated for tea type for the purpose of this review (Wang *et al.*, 2010)							
Total catechins	—	—	—	—	—	19.24 ± 10.86	—
Total theaflavins	—	—	—	—	—	7.62 ± 3.77	—
(I) Study characteristics 6 samples of pu-erh tea purchased in the Yunnan region of China: 3 green pu-erh and 3 black pu-erh, results were expressed as % (w/w) (Wang *et al.*, 2009)							
Total polyphenols	—	21.97 ± 0.48	—	—	—	—	10.31 ± 016
Total theaflavins	—	0.07 ± 0.02	—	—	—	—	0.17 ± 0.01
Total thearubigins	—	4.31 ± 1.24	—	—	—	—	5.88 ± 0.88
Total theabrownins	—	2.85 ± 0.11	—	—	—	—	9.73 ± 0.11
Gallic acid	—	nd	—	—	—	—	0.62
EGCG	—	7.69	—	—	—	—	nd

EGCG, (−)-epigallocatechin gallate; nd, not detected.

without grassy taste of green tea (Zhang *et al.*, 2011). A recent study comparing different types of tea for their hydrogen-peroxide-scavenging activity showed the following order: yellow > green > black > white > oolong tea (Gorjanovic *et al.*, 2012). A similar order of activity was also reported for total reducing power and phenolic compounds content with the exception of oolong tea being more active than white. The ability to scavenge DPPH radicals decreased in the following order: green > black > yellow > oolong > white tea.

Green Tea

Amongst all types of tea, green tea is the richest in catechins (Table 12.1). Generally, phenolic compound composition of green tea resembles that of unprocessed tea leaves. Mainly, flavon-3-ols contribute to antioxidant capacity of green tea and its sensory properties. The content of individual phenolic compounds in green tea products varies widely. There are considerable differences in green tea processing between Chinese and Japanese producers. Chinese green tea is usually dry heated in order to deactivate oxidases, whereas in the case of Japanese green tea steaming is employed. Moreover, Japanese green tea is usually shade grown, and it contains a lower content of catechins and more amino acids compared to Chinese-style tea (Ku *et al.*, 2010).

Oolong Tea

Oolong tea is the most popular in Taiwan. It is referred to as semi-fermented tea and contains a mixture of non-oxidized monomeric polyphenols and higher-molecular-weight theaflavins. There are considerable differences in the reported content of catechins in oolong tea. On one study, it was reported to be comparable to that of green tea (Table 12.1B), whereas in another study it comprised only half of that noted for green tea (Table 12.1D and F). Oolong tea can be stored for several years; so-called old oolong tea has to be stored for at least 5 years. It is believed that the longer it is stored the better it tastes. When stored for long time, tea absorbs water and has to be dried again by heating at 120°C for 10h (Lee *et al.*, 2008). Considerable increase in gallic acid content during processing of old oolong tea was noted. At the same time 50% loss in EGCG was observed, which explains gallic acid content increase.

Black Tea

Black tea is produced in the process traditionally called fermentation, which is in fact an enzymatic oxidation evoked by polyphenol oxidases native to tea leaves. As an effect of the action of polyphenol oxidases, first quinones are formed from catechins, which further react to form dimers and oligomers.

There are two possible ways of processing in order to obtain black tea. In order to accelerate the oxidation process the leaf size is reduced by rupturing the withered tea leaves using 'orthodox rollers' or 'crush-tear-curl' (CTC) machines. It aims to disrupt the cells in order to release polyphenols oxidases and to enable them to react with catechins. Black tea produced using rollers is more suitable for large-leaf-type tea, whereas the CTC method leads to obtaining small particles suitable for teabags. CTC method creates a greater surface for enzymatic oxidation. Therefore, CTC tea has lower catechin content and antioxidant capacity compared to orthodox tea (Carloni *et al.*, 2013). Black tea was reported to contain around 20-times more gallic acid compared to green tea (Table 12.1E), whereas the other studies report only two-fold differences (Table 12.1D). During black tea manufacturing the most considerable changes occur in the content of catechins. A profound loss in catechin content is observed (Table 12.2B, D, F). At the same time new compounds are formed as a result of enzymatic oxidation: theaflavins and thearubigins.

Pu-erh Tea

Pu-erh tea is still the most popular in China. It is produced from the broad-leaf variety of tea plant *Camellia sinensis* var. *assamica* in the Yunnan Province of China. It is most often commercialized in the form of compressed brick, cake log, nest, or gourd shape (Ahmed *et al.*, 2010). After harvest, the leaves follow processing similar to that of green tea to obtain the raw material for pressed green pu-erh or raw pu-erh (Figure 12.5). The deactivation of enzymes by firing is not complete for pu-erh. Therefore oxidation can occur during long storage, which develops a smooth-tasting tea. The raw pu-erh is stored for at least 10 years in clay jars, bamboo wrapping, and underground pits at a temperature close to room temperature in order to obtain tea with optimal flavor. The oxidation in pu-erh tea occurs due to the enzymatic activity of microorganisms, like *Aspergillus* sp. The older the tea gets, the more prominent its color and aroma becomes. In order to imitate this long process of pu-erh tea aging, a microbial processing was developed that leads to the production of ripened/cooked pu-erh. It is manufactured by the piling of sun-dried leaves under controlled temperature and humidity, often inoculated by selected strains, and allowed to ripen for several weeks or months. The oxidized and fermented leaves are then steamed, compressed, and dried. In China, the tea obtained in this way is called black pu-erh.

Microorganisms oxidize phenolic compounds of tea and lead to considerable loss of catechins and formation of theaflavins, thearubigins, theabrownins, and gallic acid (Table 12.1I) (Wang *et al.*, 2011; Xie *et al.*, 2009). Recently, more and more studies have been carried out to reveal the detailed composition of pu-erh tea and its antioxidant activity. The effect of processing to obtain aged and black (ripened) pu-erh on catechins content and radical-scavenging capacity was studied by Ahmed *et al.* (2010). The results indicated higher total catechin content and free-radical-scavenging capacity of green pu-erh compared to aged green and black pu-erh tea. The results confirm the statement that a decrease in polyphenolic levels and antioxidant activity of pu-erh and other teas occur with an increase in processing and aging. However, the results reported by Zhang *et al.* (2012) showed an increase in radical scavenging activity of 1-week fermented pu-erh tea and total antioxidant and superoxide anion-scavenging activity of teas fermented for up to 3 weeks in comparison to unfermented sun-dried tea. The comparison of commercially available teas of different types (Table 12.1B) revealed the lower content of catechins, glycosylated flavonols, and procyanidins in green pu-erh tea than in green and white tea (Zhao *et al.*, 2011). The content of gallic acid in ripened pu-erh was the highest among all kinds of tea studied, i.e., aged pu-erh, green, oolong, black, white, and yellow (Table 12.1A) (Zhang *et al.*, 2011).

Generally, the real effect of processing can be analyzed only when the same raw material is subjected to different processing. Most of the literature available reports on the differences in phenolic compound profile between various types of tea obtained from the market worldwide, but history of production and storage of tea is not known. The studies exploring the effect of processing leading to obtaining different tea types are still scarce. However, two papers investigating this effect have been published lately (Carloni *et al.*, 2013; Kim *et al.*, 2011a). Carloni *et al.* studied the antioxidant activity of white, green, and black tea obtained from unified raw tea leaves processed differently in the same factory to obtain two black teas (orthodox and CTC), two green teas, and one white tea. The authors reported that the total phenolic content was higher in green tea, than in white and black tea. A similar trend was observed for the content of individual catechins, except for black orthodox tea for which total catechins as well as EGCG content were higher than for white tea. As it was expected, total theaflavins content was higher in black teas, and very low in the green tea. It is worth noting that white tea also showed a significant content of theaflavins, much higher than in green tea. Theaflavins are formed because during processing of white tea oxidase is not deactivated. Accordingly, the lowest antioxidant capacity was reported for black CTC tea whereas the highest was reported for green tea.

The second study carried out aimed to identify changes during processing of unfermented tea (green tea) through oolong (20–60% fermentation) to black tea (80% fermentation) (Kim *et al.*, 2011a). The largest changes were noticed in the content of four major tea catechins EGCG, EGC, EC, and ECG. Their content decreased by more than 50%, and even up to 91% for EGC, while free gallic acid content increased. During formation of theaflavins, galloyl groups are cleaved from gallated catechins, which results in increase in gallic acid content. At the same time total phenolic content decreased, whereas total theaflavins and thearubigins increased with increasing degree of fermentation. The content of total flavonol glycosides was strongly reduced (38%) during processing, and a clear tendency of decrease with increased degree of fermentation was noticed in the case of myricetin-3-glycoside, rutin, and quercetin-3-glucosyl-rhamnosyl-galactoside. Antioxidant activity of tea infusion decreased by 20% in the course of black tea processing. The authors suggested that the loss in antioxidant capacity noted was due to degradations of ascorbic acid, and flavonol glycosides.

Home Preparation of Tea Infusions

In recent years there has been more and more research on the effect of consumer preparation on composition and activity of tea infusions (Kyle *et al.*, 2007; Lakenbrink *et al.*, 2000; Molan *et al.*, 2009; Su *et al.*, 2006). The studies have examined the different culinary methods used in the domestic preparation of tea infusions, taking into account factors such as water temperature, infusion time, stirring and dosage form, i.e., loose-leaf tea *versus* tea bag.

The extraction temperature, extraction time, water quality and water-to-tea ratio, tea particle size, extraction pH, and the number of extractions are all important factors which directly affected the efficiency of the extraction of antioxidants (Vuong *et al.*, 2011).

Water Quality

Tap water, activated carbon-adsorbed water, deionized water, distilled water, reverse-osmosis water and ultra-pure water were compared for their impact on tea quality of green tea (Danrong *et al.*, 2009). Similar radical-scavenging activity was found for tea infusions prepared with tap water, activated carbon-adsorbed water, and deionized water. However, radical-scavenging activity in the case of distilled-water-, reverse-osmosis-water-, and ultra-pure-water-prepared tea was significantly higher. Most likely radical-scavenging activity was affected by the pH differences between the different kinds of water. The effect of pH in a wide range on the content of catechins in green tea infusion was studied, and much higher content of catechins at acidic conditions of brewing ($pH < 6$) than under more neutral and basic conditions were noted. It was concluded that the effect is mainly due to different stability of catechins at different pH values, rather than to efficacy of extraction (Vuong *et al.*, 2011).

Temperature and Time

In the case of green tea it was reported that the higher temperature of brewing, the higher the reducing power of the infusion; however, no difference was noticed between extraction carried out for 10 min and 30 min (Molan *et al.*, 2009). Similarly, for black and oolong tea, the longer the time of brewing was, the higher the antioxidant activity as well as the total phenolic and catechin contents were (Kyle *et al.*, 2007). Oolong tea prepared at 95°C for 3 min had the highest scores of aroma, bitterness, astringency, and sweetness, but the total polyphenol and radical-scavenging activity in tea solutions increased with higher temperatures and longer brewing times. The brewing conditions optimal for maximal TEAC values of green tea were reported to involve 80°C for 3–5 min (Samaniego-Sanchez *et al.*, 2011). In terms of the maximal extraction of catechins per gram of green tea, the best extraction efficiency was achieved with water extraction at 80°C for 30 min (Vuong *et al.*, 2011).

Tea Leaves/Tea Bags/Tea Powder

Antioxidant activity and catechin content of green tea depends on form of powder, loose leaves, or tea bags, as well as brewing conditions (Komes *et al.*, 2010). Powder and bagged green tea allowed the extraction of more phenolic compounds than leaves, regardless of the extraction temperature. Total flavan-3-ols amounted to around 1 g/l, whereas for loose-leaf teas ranged from 0.35 to 0.9 g/l for different tea brands. The highest concentration of flavon-3-ols was obtained for extractions performed at 80°C for 5 min (powder), 15 min (bagged), and 30 min (loose leaf). Generally, higher antioxidant activity was noted for tea powder and tea bags than tea leaves. Antioxidant activity was positively influenced by increased extraction time and temperature. However, other studies showed that the extraction efficacy of phenolic compounds from white (Rusak *et al.*, 2008) and green tea (Samaniego-Sanchez *et al.*, 2011) was not significantly affected by form of loose leaf *versus* tea bag.

Serving Additives

Addition of milk can reduce antioxidant potential of black tea (Ryan and Petit, 2010). In the case of green tea the results are contradictory: The addition of milk to powdered Matcha green tea mildly decreased its antioxidant activity, whereas for two other types of tea (Gyokuro and Twinings) the opposite effect was observed (Komes *et al.*, 2010). The antioxidant activity of green tea was enhanced after addition of lemon juice, which might be explained by the synergistic effect of ascorbic acid on the polyphenols as well as increased stability of polyphenols due to lower pH.

Production of Ready-to-Drink Tea Beverages

Tea drinks are becoming more and more popular worldwide. Tea beverages are produced on the basis of tea extracts or tea infusions prepared as a part of beverage manufacturing process and available on the market bottled or canned (Cordero *et al.*, 2009). Generally, very low content of catechins was noted for most tea beverages studied in comparison to tea-cup- or tea-pot-prepared tea. Fourteen brands of canned and bottled green tea drinks were analyzed by Chen *et al.* and in 10 of them the total catechin content was lower than 3 mg/100 ml (Chen *et al.*, 2001). Only in three of all analyzed tea drinks produced in Japan was the content of catechins about 30 mg/100 ml. Moreover, the profile of phenolic compounds in tea beverage differs from that of original tea. For green tea beverages the content of GCG considerably exceeded that of EGCG. GCG is a product of epimerization of EGCG, which might occur due to temperature treatment. One of the steps during processing of tea beverages is sterilization, at a temperature of 120°C for several minutes. After heating for 20 min, only 76% green tea catechins remained in water solution, and the stability was shown to be pH dependent (Chen *et al.*, 2001). Also heating for as little as 1 min at 120°C caused loss of 15 or 36% of total catechins for steamed and roasted green tea, respectively (Wang *et al.*, 2000). Moreover, it was suggested that steamed green tea leaves are a better starting material than roasted tea for production of bottled or canned tea beverages since they contain more flavanols (Wang *et al.*, 2000). In this way, products rich in antioxidants and with good sensory qualities can be obtained.

Eighteen brands of canned and bottled drinks (10 green teas, two oolong teas, six black teas) available in Hong Kong were studied for their catechin profile and antioxidant activity. Epimers dominated in oolong and black tea beverages, whereas for green tea similar content of both forms of catechins was found (Xu *et al.*, 2004).

The content of catechins in fermented tea beverages of eight brands available on the Italian market was highly variable (Cordero *et al.*, 2009). The sample richest in catechins, (>200 μg/ml) was produced from tea leaf infusion. It was suggested that products obtained by an industrial tea leaf infusion process are a better source of flavan-3-ols than those produced from reconstituted tea extracts. However, the impact of the quality of raw material, additives, thermal treatments, formulation, and packaging can not be neglected.

STORAGE OF TEA LEAVES AND TEA BEVERAGES

Tea leaves have a considerably long shelf-life due to their low moisture content. In the case of pu-erh tea and old oolong tea, long storage is even necessary for the development of desirable taste and aroma. However, for other tea types, storage for an extended period of time can lead to loss of quality of the product. It was reported that tea catechins are not stable during long-term storage. During 6 months' storage of green tea bags in their original containers at room temperature in the dark, the average content of EGCG decreased by one-third and ECG decreased by half. This suggests that ECG may be more susceptible to degradation than EGCG (Friedman *et al.*, 2009). Storage of black tea for up to 12 months can affect theaflavins and thearubigins content (Thomas *et al.*, 2008). The loss of theaflavins was greater for orthodox black tea (around 37%) than for CTC (22%), whereas for thearubigins the relation was opposite. It was indicated that γ-irradiation could reduce this deterioration to a great extent. The main factors influencing tea quality and catechin stability during storage are light, oxygen, and temperature. The optimal storage conditions for green tea powder to maintain catechin stability were indicated by Li *et al.* as ambient temperature and relative humidity below 43% or in a freezer at relative humidity below 60% (Li *et al.*, 2011).

In the case of ready-to-drink beverages, the great influence of packaging material on storage stability is evident. The shelf-life of the products is usually 12 months; nevertheless, product aging during that time was found to be extensive. Storage stability of green tea infusions depending on packaging material used (glass, PET, and retortable pouch) was evaluated by Kim *et al.* (2011b). Tea drinks were stored in the absence of light at a temperature of 3°C and catechins were found to be stable up to 6 weeks, and after that time the degradation appeared to a greatest extent in retortable pouch > PET > glass (Kim *et al.*, 2011b). The antioxidant capacity of green tea infusions decreased by 18, 14, and 30% in glass, PET, and retortable pouch, respectively, during the 12 weeks of storage. The differences were caused most probably by different oxygen permeability of packaging materials. When PET, polystyrene glass, and cans used as packaging for fermented tea drinks originating from the same batch were compared, the highest storage stability was found for drinks in cans (Cordero *et al.*, 2009). Nevertheless, also in cans there was a 50% loss of catechins. It was reported that cold storage at 4°C of tea beverages in PET bottles ensures a slower decrease in catechin content in white, black, and green teas (Nekvapil *et al.*, 2012).

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