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Review Paper / Derleme Makale

Factors Affecting Polyphenol Content and Composition of Fresh and Processed Tea Leaves

Nihal Turkmen[1], Ferda Sarı[2], Y. Sedat Velioglu[2]

[1]Uludag University, Vocational School of Technical Sciences, Görükle Campus, Nilüfer, Bursa
[2]Ankara University, Faculty of Engineering, Department of Food Engineering, Ankara, Turkey
E-mail: velioglu@eng.ankara.edu.tr

ABSTRACT

Tea has been consumed literally for thousands of years and is recognized as a major source of polyphenols in human diet. Polyphenols comprising 20-40% of dry matter in young tea shoots are very important constituents of tea from an intrinsic quality point of view. They are responsible for the colour, flavour and brightness of tea. Recently, tea polyphenols have become a subject of intense studies by scientists throughout the world because of their health beneficial effects and potential uses. On the other hand, there are numerous factors affecting polyphenol structure of tea leaves, such as tea leaf variety, harvesting season, climate, processing method and analytical method. This review is presenting factors affecting polyphenol content and composition of in fresh and processed tea leaves, i.e., green and black teas.

Key Words: Leaf handling, Leaf age, Leaf variety, Harvest methods, Season, Processing

Taze ve İşlenmiş Çay Yapraklarındaki Polifenol İçeriği ile Dağılımına Etki Eden Faktörler

ÖZET

Binlerce yıldır tüketilmekte olan çay, diyette polifenollerin ana kaynaklarından biridir. Polifenoller taze çay yapraklarında kuru maddenin %20-40'ını oluşturur ve çay kalitesinin en önemli ögelerinden biridir. Polifenoller çayın renk, lezzet ve parlaklığından sorumlu bileşiklerdir. Son yıllarda çayın sağlık üzerindeki olumlu etkilerinin ve potansiyel kullanım olanaklarının ortaya çıkmasından sonra çay polifenolleri üzerinde daha yoğun olarak çalışılmaya başlanmıştır. Çayın polifenol yapısını etkileyen çok sayıda faktör bulunmaktadır. Bunların başlıcaları çay yaprağı varyetesi, hasat dönemi, işleme yöntemi ve uygulanan analiz metotlarıdır. Bu makalede taze çay yapraklarının ve bunlardan işlenen yeşil ve siyah çayların polifenolleri üzerine etki eden faktörler irdelenmiştir.

Anahtar Kelimeler: Yaprak toplama, Yaprak yaşı, Çay yaprağı varyetesi, Hasat yöntemi, Sezon, Proses

INTRODUCTION

Tea is the most widely consumed beverage worldwide and has become an important agricultural product [1]. It is generally consumed in one of three forms: green, oolong and black. Approximately 3.0 million metric tons of dried tea is produced annually, 20% of which is green tea, 2% is oolong, and 78% is black tea [2]. Brewed tea provides a significant source of phenolic compounds in the diet [3-5]. These compounds constitute up to 30% of the tea solids by weight [6-7]. Recently, tea is considered as a functional food because of reported beneficial effects on human health, in particularly as an antioxidant [8-14], antimutagenic [15-19], anticarcinogenic [6, 20-22] and antibacterial [23-27]. Tea is now consumed throughout the world not just as a popular beverage, but, because its extracts have been prepared in a variety of physical forms, for example, strong infusions, soft extracts and powders, it is now widely available in a range of food, beverage, and toiletry and cosmetic products [28]. The use of tea extracts in edible oil systems [11, 29-30] and cooked muscle foods [31-32] reduces the rate of peroxide accumulation, improving product stability.

Extensive studies have focused on health effects of green tea (GT). However, Leung *et al.* [33] reported that theaflavins (TFs) in black tea (BT) possessed at least the same antioxidant potency as catechins in GT and the conversion of catechins to TFs during fermentation in making BT did not alter their antioxidant acitivity significantly. Hodgson *et al.* [34] showed that insignificiant differences in antioxidant activity were found between BT and GT. Furthermore, BT had greater antimutagenic activity [16] and was more efficient chemopreventor against reactive oxygen and nitrogen species in comparison to GT [21].

The main polyphenol components of fresh and GT leaves are flavan-3-ols the so-called catechins which include epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG), epigallocatechingallate (EGCG) and catechin (C) [16]. Flavonols and phenolic acids like gallic and cinnamic acids are found in fresh and GT leaves in trace amounts [35]. Main flavonols in tea leaves are quercetin, myricetin and kaempferols which are predominantly present as glycosides [28, 36]. During BT processing, the endogenous flavan-3-ols in fresh GT leaves undergo various oxidative reactions to produce stable components that include theaflavins (TFs), thearubigins (TRs) and other polymerization products [37]. Phenolic compounds of tea are closely associated with the sensory properties and hence the quality of a tea brew [39-39]. Catechins are colourless, water-soluble compounds and impart bitterness and astringency to GT infusion [36, 40]. In BT, orange-coloured TFs and brownish TRs are responsible for both the reddish color and the astringent effect [4, 28, 41]. TFs are also considerably important in predicting the quality of BT [42]. Tea polyphenols are strongly influenced by various factors such as variations in leaf variety, harvesting season, climate, processing method and analytical method [12, 36, 43-44]. Therefore, all these factors should be taken into consideration for the comparison of the reported results on tea polyphenols.

The aim of this work is to review factors that affect in polyphenols in fresh and processed tea leaves in the view recent findings.

POLYPHENOLS IN FRESH TEA LEAVES

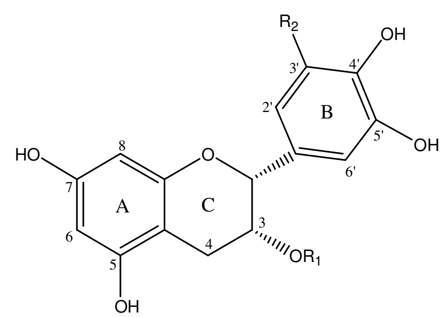
Phenolic compounds in fresh tea leaves are composed of predominantly flavan-3-ols (catechins) and flavonol glycosides and phenolic acids [38, 45]. As seen in Table 1, the most significant groups of the polyphenols are catechins, which are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings (Figure 1) [46], and the major tea catechins are EGCG, EGC, ECG, EC, gallocatechin (GC) and C [47]. Catechins are generally water soluble and colorless compounds [48].

In fresh tea leaves, either EGC or EGCG was the dominant flavan-3-ol [49]. For 40 African tea clones, EGCG was the most abundant flavan-3-ol, which was followed by EGC. The other flavan-3-ols (C, EC and ECG) were much less abundant [42]. Similarly, Yao et al. [38] found that EGCG was the main flavan-3-ol in Australian fresh tea shoots, constituting up to 115 mg g^{-1} of tea shoots on dry basis, followed by EGC (47.9 mg g^{-1}), ECG (40.7 mg g^{-1}) and EC (17.5 mg g^{-1}), respectively. In various Taiwan fresh tea leaves, the content of each catechin was in the following order: EGCG>EGC>ECG>EC>C [47]. The same order was also reported by Aucamp *et al.* [50]. The high levels of the first four catechins, in particular EGCG, are very important to BT quality [35]. The reason is that these catechins lead to the formation of the major TFs responsible for BT quality attributes; however, other catechins are not precursors of the TFs [42, 51].

Table 1. Phenolic composition of the fresh tea flush (adapted from [48])[1]

Component	Dry Weight (%)
Flavan-3-ols (catechins)	18-32
EGCG	9-14
EGC	4-7
ECG	2-4
EC	1-3
GC	1-2
C	0.5-1
Minor catechins	0.4-1
Flavonol glycosides	3-4
Proanthocyanidins	2-3

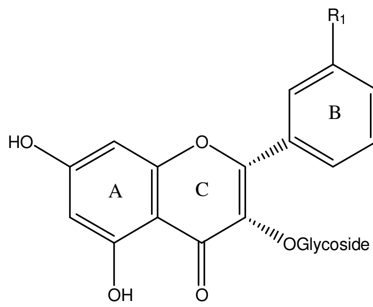
[1)] Please refer to text for abbreviations



Epicatechin: $R_1 = R_2 = H$
Epigallocatechin: $R_1 = H$; $R_2 = OH$
Epicatechin-3-gallate: R_1 = Galloyl; $R_2 = H$
Epigallocatechin-3-gallate: R_1=Galloyl; R_2=OH

Figure 1. Chemical structure of major flavan-3-ols present in tea leaves

Flavonols are predominantly present as glycosides rather than as their aglycones (non-glycosylated forms) [28, 36], with one, two or three carbohydrates attached to flavonol hydroxyl groups (Figure 2) [52]. They are structurally more stable than catechins [53]. There are three major flavonol aglycones in the fresh leaf: kaempferol, quercetin and myricetin. The glycosidic group may be glucose, rhamnose, galactose, arabinose, or rutinose [48]. Polyphenols in fresh tea leaves vary depending on the various factors. These are:



Kaempferol glycoside: $R_1 = R_2 = H$
Quercetin glycoside: $R_1 = OH$; $R_2 = OH$
Myricetin glycoside: $R_1 = R_2 = OH$

Figure 2. Chemical structure of flavonol glycosides present in tea

Leaf Handling

After harvesting, leaves must be transported with great care to the factory immediately to avoid any crushing or other damage. Rough handling may nullify the efforts careful plucking. This is because any leaf damage may initiate undesirable chemical and biochemical reactions at this early stage [35]. Green leaves start withering process from the moment it is plucked from the tea bush. Overheating leaves causes more quality loss than physical leaf damage. Poor-handled leaves turn red due to the oxidation of catechins. Black tea made from red leaves have lower TFs content, which means reduction in quality [54].

Leaf Age

Shoots in tea manufacture are composed of a bud and three leaves on average. The distribution of the catechins within a shoot varies with the age of leaves [55]. According to Lin et al. [47], the content of total catechins is in the following order: young leaves (5.86%)>old leaves (2.15%)>stem (0.85%), and the content of individual catechins also varies with the age of leaves (Table 2). Similar results were reported by Caffin *et al.* [35]. On the other hand, Chen *et al.* [56] showed that EGCG decreased markedly between the bud and the fourth leaf, and EGC increased markedly between the bud and the sixth leaf; however, EC showed no significant change from the bud to the sixth leaf. Furthermore, Lin et al. [57] found that the levels of EGCG, EGC and EC in old tea leaves were higher than those in young leaves for different kinds of tea samples except for one kind of tea. With respect to BT manufacture, the TFs content reached a maximum with two leaves and a bud. But, additional leaves caused decrease in TFs levels, probably due to lower levels of polyphenols and polyphenol oxidase activity [58].

Table 2. Various catechin contents of different parts of of fresh tea plant (Wun-Yi variety) (adapted from [47]))

Leaf component	Type of Catechins (% sample weight)				
	EGCG	EGC	ECG	EC	C
Young leaves	2.83	1.29	1.31	0.44	0.14
Old leaves	1.02	0.84	0.39	0.28	0.07
Stem	0.32	0.38	0.11	0.20	0.03

Seasonal variation

The content and distribution of catechins in fresh tea leaves may vary with the harvesting season. In a study by Caffin *et al.* [35], the levels of EGCG and ECG, the main catechin gallates in tea flushes, were higher in the leaves harvested in warmer months in Australia. In contrast, EGC was at higher levels in cooler months. The authors suggested that fresh leaves harvested during warmer months contained higher catechin gallate levels and had greater potential for making high quality BT than those harvested at other times of the year. Lin *et al.* [47] found that the amount of individual catechins in fresh tea leaves was higher in summer (avg. 5.25%) than in spring (avg. 3.77%) because the growth rate and metabolic activities of the leaves were higher in summer. Additionally, harvesting season can enhance the health effect of tea. Investigation the effect of manufacturing season on the antimicrobial activity of tea Chou et al. [59] found that the catechin content in tea leaves varied with season; and the tea product made from tea leaves harvested in summer exhibited the strongest antimicrobial activity due to the highest content of catechin.

Harvest method

Tea leaves are harvested by hand or shear plucking or mechanically from the field. The content of polyphenols in tea leaves varies depending on the harvest methods. In general, hand plucked fresh tea leaves contain higher levels of phenolic compounds than mechanically harvested leaves, which are usually more mature [35], and shear plucked leaves, which are more coarse and injured. Furthermore, tea made from hand-plucked leaves was found to be better than that obtained from shear-plucked leaves with respect to organoleptic evaluation [60].

Tea leaf variety

The content of individual catechins in fresh tea leaves varies with the tea clones [61-63]. Caffin et al. [35] reported that the distribution pattern of polyphenols varied a great deal among tea clones whereas the patterns appeared to be more or less fixed within a clone. Similary, total polyphenols content in green leaves showed minimal variations within a same clone but significant differences among clones [49]. In a study by Wright et al. [64], among the 20 good and 20 poor quality tea clones, significant difference was observed in the contents of EC, EGC and ECG, which are correlated with BT quality. The flavan-3-ol composition of GT leaves varied more among Kenyan tea clones, from 52.25 to 73.75 mg equivalent catechin g^{-1} fresh weight, and either EGC or EGCG was the dominant flavan-3-ol present in fresh tea leaves [49]. Owuor and Obanda [63] reported that the distribution of the individual flavan-3-ols in green tea leaves may be more critical to theaflavin formation than total amounts of flavan-3-ols. Significant differences were also observed in individual theaflavin compounds except for theaflavin digallate contents in BTs from good and poor quality tea clones [42]. Besides polyphenols content, the content of PPO (polyphenol oxidase) enzyme content showed variation among different tea clones [61], which also influenced the final BT quality, and the ratio between PPO and its substrate (catechins) was used to characterize the potential quality of tea clones [65]. Tea clones may have different fermenting characteristics (e.g. slow or fast fermenting). Poor fermentability of some clones were still green after 90 min fermentation, implying that they would be more suited to GT production but in BT production slow and fast fermenting clones were mixed, and the extent of clone mixing was determined by the balance between yield (production) and quality (value) desired [66].

POLYPHENOLS IN GREEN TEA

Green tea, the non-fermented tea, is produced by inactivating enzymes in tea leaves at the onset to prevent oxidation of the leaf polyphenols [6]. A distinctive feature of GT processing is that leaves are never subjected to any possible condition of fermentation. Freshly harvested leaves are steamed immediately at 95-100°C for 35-45 seconds to inactivate the enzymes, especially polyphenol oxidase. The steamed leaves are then rolled to make a slender pickle form followed by drying in current air of moderate temperature. The rolled and dried leaves are finally fired (roasted) and cut to prepare the final products by tea dealers [67].

Chemical compounds of GT including polyphenols differ very little from those fresh leaves [44]. Green tea polyphenols are composed of mainly catechins forming nearly 30% of the dry weight of the tea [3, 5, 36, 44]. Catechins are responsible for the unique taste of green tea, especially astringency [68]. EGCG is the most abundant catechin in GT, which is followed by EGC, ECG, EC, catechingallate (CG), GC and C, respectively [43, 47, 69-71]. However, it has been reported that the content of catechins was in the order of EGCG>EGC>EC>ECG>GC [72, 73] or EGCG>EGC>GC>ECG>C>EC [74]. The authors [72-74], also, indicate that EGCG is the dominant catechin in GT. The high concentration of EGCG can cause the greatest contribution to antioxidant activity of the GT nearly 30% of the total antioxidant activity [75]. This activity is attributed to the presence of an ortho-trihydroxyl group in B ring and a galloyl moiety at position 3 (Figure 1) [8, 46, 75]. Similar results were reported by Lin *et al.* [47]. However, in other investigations, it was also stated that EC [76] or ECG [33] had the highest antioxidant activity among catechins. These different results are attributed to different test systems [47]. Another group of polyphenols in GT is flavonols, which make up to 3% of dry weight of tea leaves and predominantly present as glycosides [28, 36]. Main flavonols in tea are myricetin, quercetin and kaempferol [77, 78]. In general, quercetin content was the highest in GT, which is followed by kaempferol and myricetin, respectively. Only quercetin glycosides were reported to exibit antioxidant activity [75]. But, antioxidant activity of quercetin aglycone is reduced by its sugar moiety [46], because quercetin includes the 3-hydroxyl group in the C ring, which enhances its antioxidant activity [79].

The content and the composition of GT catechins may vary with the species of tea plant, the season for harvesting [80], the conditions of processing [68], the variety of tea, the analytical method applied, especially extraction conditions, leaf age [38, 43, 81] and climate and growth conditions [44, 82]. In the manufacture of green tea, harvested fresh leaves are immediately subjected to heating or steaming treatment to inactivate the enzymes contained, especially PPO [67] which is the key treatment for processing green tea [83]. PPO inactivation inhibits the oxidation of catechins to form teaflavins and tearubigins [68]. However, during green tea processing, the content of total tea polyphenols may decrease to some extent (*ca.* 15%) in comparison with fresh leaves due to their oxidation, hydrolysis, polymerization and transformation [83]. Furthermore, tea catechin composition changes remarkably. For example, gallated catechins convert into non-gallated catechins through hydrolysis under humid and heating conditions [83]. Nishitani and Sagesaka [70] investigated the content of individual catechins of different types of GT (Table 3), and noted that catechin contents of the Matcha tea were lower than in the Sencha, probably due to shade cultivation of its raw leaves, which lowers biosynthesis of catechins. This phenomenon is related to the activity of phenylalanine-ammonialyase, which is a key enzyme in the biosynthesis of catechin B ring. When tea plant is covered (blocking out light), this enzyme activity decreases rapidly [84]. Perva-Uzunalic et al. [71] investigated the extraction efficiency of catechins of GT using different extraction set-ups, different aqueous and pure solvents and different temperatures and times. They demonstrated that the content of major catechins in GT extracts varied from approximately 280-580 g kg^{-1} dry extract, with extraction efficiencies of major catechins varying from 61% to almost 100%. The effect of different extraction procedures (e.g. different solvents and times) on the catechin concentrations of GT was also shown by Sharma *et al.* [7] (Table 4). Furthermore, in a study by Goto *et al.* [69], Matcha and Sencha teas with two different grades were analyzed for catechin, and lower grade teas contained more catechins than higher grade ones.

Table 3. Comparison of catechin contents of Japanase and Chinese green tea samples (adapted from [70])

Type of catechin	Japanase GT (% of dry weight)		Chinese GT (% of dry weight)
	Sencha	Matcha	Gunpowder
EGCG	8.71	5.24	4.91
EGC	6.27	2.88	1.97
ECG	1.55	0.86	1.41
EC	0.97	0.45	0.43
GCG	0.09	<0.003	0.05
GC	<0.007	<0.007	<0.007
CG	0.02	<0.007	<0.007
C	<0.006	0.01	0.05

Table 4. Effect of extraction condition on the concentration of GT catechins (adapted from [7])

Extraction condition		Concentration (mg g^{-1} of dry weight)			
		EGC	EC	EGCG	ECG
Solvent	Methanol, 70%	33.0	9.5	59.2	3.58
	Methanol	12.5	1.0	36.0	2.0
	Acetone	4.0	1.0	13.0	1.5
Extraction time (min) *(with 70% methanol)*	10+10+10^{2}	33.0	9.5	59.2	3.58
	30	1.5	0.30	50.0	2.50
	10	nd$^{1)}$	nd	30.0	2.30
Ratio of solvent *(with 70% methanol)* (mL):solid(g)	250	12.11	nd	36.11	18.17
	150	15.13	0.091	50.67	11.32
	50	21.90	0.97	86.89	5.01

[1]nd: not detected, [2]three step extraction

POLYPHENOLS IN BLACK TEA

Black tea differs from GT in production process which influences the chemical constituents and taste of tea [12]. Black tea is manufactured in four distinct stages; withering, maceration or rolling, fermentation and drying (firing) [54]. During withering, the leaves take on a form facilitating the rolling process. This process results in disrupting the cell structure of the leaves, and the fermentation process then begins [85]. The fermentation stage involves an enzyme-catalysed oxidative reaction to the colored phenolic compounds. These brown coloured pigments formed during the fermentation process are the products of catechin oxidation [48]. The fermentation process is stopped by a blast of hot air in a “tray dryer” for orthodox manufacture or a “fluid bed dryer’’ for CTC (crush-tear-curl) to reduce the moisture content to 2.5% [86].

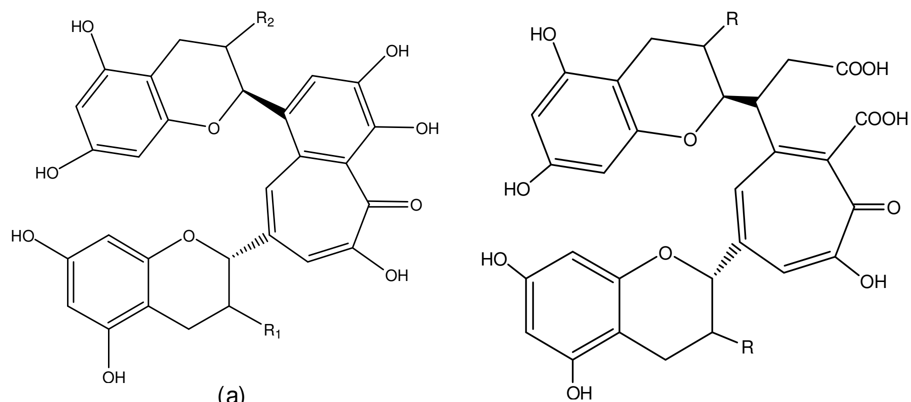
Of tea polyphenols, flavan-3-ols are significantly reduced during the fermentation process and are, therefore, either absent or present in low levels in black tea [75] (Table 5). In a study of Lin et al. [87] about the effect of fermentation on the levels of tea catechins, high degree (85.00%) of fermentation for black tea resulted in a considerable decrease in EGCG (95.36%), EGC (73.61%) and EC (92.59%). The authors also reported that ECG, C and GCG were not detected after the fermentation process.

The major catechin oxidative products are TFs and TRs (Figure 3) [48] which give BT its characteristic color and taste [4,44,88]. The synthesis and the molecular structure of the TFs which comprise 0.3-2% of the dry matter of BTs [28] are well described [89]. TFs composition of BT is dominated by four TFs: simple theaflavin (TF-f), theaflavin-3-gallate (TF-3-g), theaflavin-3’-gallate (TF-3’-g), theaflavin-3-3’-digallate (TF-3, 3’-dg) [51]. A characteristic element of TFs structure is the seven-member benzotropolone ring. Other benzotropolone compounds in addition to the TFs were also identified in black tea, but in considerably smaller amounts [85]. However, TRs, constituting 30-60% of soluble solids of BT, are still poorly understood [42,90] since they are heterogeneous polymers of flavan-3-ols [41,44,91]. Three groups of TRs are defined: (i) SI, soluble in ethyl acetate; (ii) SIa, soluble in water and diethyl ether; (iii) SII, soluble in water [64, 92].

Table 5. Catechin concentration in different tea samples (adapted from [73])

Type of catechin	Concentration (mg 100 mL^{-1})[1]			
	Gunpowder green tea	Sencha green tea	Keemun black tea	Sri Lanka black tea
GC	2.57	2.81	0.4	1.57
EGC	29.7	36.2	0.9	1.84
C	0.69	1.41	nd	0.5
EGCG	32.6	28.8	0.95	1.16
EC	5.58	9.54	nd	1.45
GCG	0.51	1.02	nd	nd
ECG	4.26	4.92	1.19	2.92
Total catechins	76.00	84.60	3.44	9.43

[1]: 3 g of tea leaves were brewed with 150 mL of boiling distilled water for 5 min, nd: Not detected



(a)

Theaflavin: $R_1 = R_2 = OH$
Theaflavin-3-gallate: R_1 = Galloyl; R_2 = OH
Theaflavin-3'-gallate: R_1 = OH; R_2 = Galloyl
Theaflavin-3,3'-digallate: $R_1 = R_2$ = Galloyl

(b)
R=Galloyl or other groups

Figure 3. Chemical structure of theaflavins (a) and thearubigins (b)

Significant correlations between tea liquor sensory characteristics and total or individual TFs were reported in the literature [37, 41, 51, 63, 64, 93]. BT has been regarded, because of its low content of monomeric polyphenols, as having significantly weaker antioxidative properties compared to green tea. However, partial polimerization and other alterations occurring during fermentation of tea leaves did not diminish the antioxidative properties of black tea [85]. As mentioned before, TF compounds make an important contribution to antioxidative properties of BT. However; their effectiveness varies with each of the individual TFs. For example, Miller *et al.* [94] found that radical scavenging activity of these compounds decreased as follows: TF-digallate>TF-3'-g = TF-3-g>TF-f. Similar results were also obtained by Leung *et al.* [33] who demonstrated that antioxidant activity of individual TFs using human LDL oxidation as a model was in the following order: TF-digallate>TF-3'-g>TF-3-g>TF-f.

According to Wang and Helliwell [53], there was a difference in flavonol contents of different green and black teas, which was attributed to the different tea varieties, geographic location or agricultural conditions (Table 6). However, the difference was not generally from tea processing, indicating that flavonols were not affected by PPO. This was also reported by other studies [85, 95]. Additionally, Stewart et al. [75] reported that flavonols were present in similar levels in GT and BT indicating that they are relatively stable during fermentation process. The authors, also, noted that three quercetin conjugates present in highest amounts in teas exhibited antioxidant activity whereas no antioxidant activity was associated with kaempferol conjugates. Hertog et al. [95] found that in black tea infusions quercetin (10-25 mg L^{-1}) was the major flavonoid, followed by kaempferol (6.3-17 mg L^{-1}) and myricetin (1.7-5.2 mg L^{-1}), respectively. Similarly, in the infusions of a range of black tea and its products seven quercetin, five kaempferol and two myricetin glycosides were present and quercetin glycosides were the major group in all samples (50-76% of the total) except in the China black tea Keemun where they represented only 36% of the flavonols. Kaempferol glycosides were also significant components in all teas [96].

Table 6. Content of flavonols in the leaves of different tea samples (adapted from [53])

Tea leaf	Concentration (g kg^{-1} dry leaves)		
	Myricetin	Quercetin	Kaempferol
Green tea			
Gunpowder	1.59	4.05	1.56
Zhejiang	0.93	2.84	2.38
Sencha	1.32	3.75	3.31
Longjing	0.83	1.79	2.41
Black tea			
Qimen	0.24	1.04	2.31
Ceylon	0.52	3.03	1.72

Main factors affecting the content and the composition of polyphenols in BT (e.g. clones, geographical locations, and climate) were similar to those for fresh leaves and GT. For instance, according to Ding *et al.* [97], the content of polyphenols in BT markedly varied with different producing areas such as Kenya, India and China (Table 7). Additionally, BT processing stages such as withering, rolling and fermentation play an important role in the structure of TFs. Since the TFs are very important to quality [98], the factors affecting TFs content of BT can also be considered important quality parameters. The effects of processing stages on polyphenols of BT are as follows:

Table 7. Catechin and TF contents of in black teas from different orijin of production (adapted from [97])

Type of catechin or theaflavin	Concentration (g $100g^{-1}$ dry matter)		
	Assam Broken	Kenya	China
EGCG	1.21	0.84	0.36
EGC	2.72	2.21	1.23
ECG	1.03	0.74	0.28
EC	0.14	0.19	0.08
C	0.21	0.22	0.07
TF-f	0.16	0.11	0.07
TF-3-G	0.12	0.06	0.03
TF-3'-G	0.24	0.18	0.11
TF-3,3'-DG	0.31	0.12	0.08
Total TFs	0.83	0.47	0.29

Withering

The withering is actually partial drying of tea leaves, and it is carried out to condition the leaf physically for the next operation in rolling either in orthodox or any other rolling machine [99]. Shortly withered leaves produce tea with more brightness and briskness due to increase in TF formation [54]. During withering, excessive loss in green leaves moisture content (e.g. long time or high temperature) causes reduction in PPO activity of the leaves [100]. For example, as the moisture in the leaf decreases from 72.5 to 64.8 % TF levels in final BT declines by 16% [54]. Obanda et al. [37] reported that TF levels decreased with an increase in wither duration and so, the longers the wither duration the less bright the BT liquors became. In addition, Sud and Baru [101] reported that the lowest values for TF and brightness were observed in rainy season teas, probably due to inconsistent and low degree of withering which decrease the PPO activity. On the other hand, a comparison of the fermentability of normally withered and freeze-withered leaves was carried out by Muthumani and Kumar [102], and it was reported that TF levels were higher while the liquor was brighter in leaves freeze-withered for 4h.

Rolling

Black tea is usually processed in two different ways, *orthodox* and *CTC* processing, and the main difference between them is the rolling phase of the fermentation process [103]. During rolling of tea leaves, leaves are macerated and cell structures are disrupted, which brings various enzymes into intimate contact with their substrates, the polyphenols [35, 65]. Enzymatic oxidation of catechins starts immediately after the leaves are damaged [88]. CTC teas, in general, have higher TF levels compared to orthodox teas because of quick and severe maceration of the leaf cells and rapid polyphenolic oxidation [104-105]. In other words, total catechin content of the CTC-manufactured black teas is lower as a function of the greater leaf disruption [106].

Fermentation

Fermentation is one of the critical steps in BT manufacture because of the significant chemical transformations occuring during this phase [58,107]. In fermentation stage, an enzymatic oxidation of the polyphenols, especially tea catechins, takes place, leading to the formation of TFs and TRs, which are responsible for the characteristics of BT liquors [93]. In BT production process, about 75% of catechins contained in tea leaves undergo enzymatic transformation consisting in oxidation and partial polymerization [85]. Enzymes involved in oxidation of catechins are mainly PPO and peroxidase (POD) [42]. PPO catalyzes oxidation of the *o*-dihydroxy and *o*-trihydroxy phenyl "B" rings of the substrate flavan-3-ols into highly reactive *o*-quinones derivatives [92]. The quinones from the oxidation of B-ring dihydroxylated catechins (EC and ECG) condense with quinones arising from the B-ring trihydroxylated catechins (EGC and EGCG) to give four different TFs (Equations 1-4) [41].

EC + EGC	→	TF-f	(1)
EC + EGCG	→	TF-3-g	(2)
ECG + EGC	→	TF-3'-g	(3)
ECG + EGCG	→	TF-3,3'-dg	(4)

Another enzyme, POD, is responsible for the oxidative breakdown of TFs and the generation of TRs as well [92]. On the other hand, Sang et al. [91] showed that POD oxidized individual catechins into different types of dimers in the presence of hydrogen peroxide (H_2O_2). PPO activity of tea leaves widely varies with various parameters such as clone type, season, shoot component and process (Table 8). Lopez *et al.* [65]

investigated variations in the substrate level and enzyme activity of tea clones, and found that catechins and PPO showed variation, which influenced the final BT quality. According to the authors, the clones which recorded higher values of enzyme substrate ratio showed significantly higher levels of TFs.

Table 8. Variation in polyphenol oxidase (PPO) activity of tea leaves (adapted from [100])

Parameter	Leaves	PPO[1]
Clone	UPASI-3	46
	UPASI-9	36
	UPASI-17	29
	SA-6	14
	TRI-2024	39
	CR-6017	41
Shoot component	Bud and 1st leaf	39
	2nd leaf	34
	3rd leaf	31
	4th leaf	29
	Stem	9
Season	January-March	29
	April-June	38
	July-September	33
	October-December	35
Process	Fresh leaf	37
	Injured leaf	33
	Withered leaf	
	Soft	33
	Normal	30
	Hard	28
	Rehydrated	36
	Rolled leaf	35
	Fermented leaf	
	15 min	39
	30 min	39
	45 min	34
	60 min	21
	Fired leaf	
	1 min	24
	10 min	9
	20 min	4
	30 min	2
	Made tea (6-months old)	0

[1]: 1 unit= 1 µmol of catechin oxidized/min/g acetone powder.

During fermentation, the levels of individual TFs declined with increase in fermentation temperature. For example, BTs fermented at 20°C were more brisk and/or astringent than those made at 30°C due to higher amounts of individual TFs [41]. TFs are thought to partially transform into TRs during fermentation process [101]. Increasing fermentation duration may lead rise the levels of TFs at 22°C [37] and 26-28°C [58] fermentation temperatures quadratically. Muthumani and Kumar [108] reported that during fermentation, the maximum TF level occurred at the 45th minute of fermentation and as the time progressed, TF declined slowly. Adjusting the pH of the fermenting tea from 5.5 to 4.5-4.8 resulted in an increase in TF levels and a reduction in TR levels, which may be due to lower turnover rate of formed TFs to TRs [35]. In an *in vitro* fermentation system, low oxygen (O_2) concentration inhibited the formation of TFs [55]. Similar results were also reported by Biswas et al. [109]. PPO enzyme oxidizes the gallated catechins (ECG and EGCG) more efficiently, resulting in their disappearance more rapidly compared to non-gallated species (EC and EGC). At the same time the gallated TFs are generated faster than the non-gallated ones [89]. Of these four catechins, the effects on PPO enzyme are different. While ECG and EGCG were responsible for enzyme inhibition others were not [42,55], because they had higher molecular weights and flexibility [63].

Drying

Drying is the final stage of tea processing, blocks the enzyme activity and reduces the moisture content to ~3 % in order to have better shelf-life of the final BT [110]. Caffin et al. [35] demonstrated that drying process led to decrease or slightly increase in individual phenolic compounds of fermented tea leaves depending on harvest season and type of phenolic compound. Also, residual activity of PPO was noted in the dried BT, which was expected to alter the quality of end-product on storage [100]. On the other hand, the size of the leaves to be dried which is the largest in orthodox, smaller in rotorvane and the smallest in CTC manufacture determines the period of drying and the texture of the tea produced. Larger leaf particles require high temperature or a longer period of drying, which favours the conversion of TFs to TRs [104].

CONCLUSIONS

The polyphenols composition of fresh and processed GT leaves is similar but that of BT differs from these two because of oxidation and polymerization of catechins during processing. Studies showed significant variations in the content and composition of polyphenols in tea leaves depending on the various factors such as tea leaf variety, growth condition, climate, processing and analytical methods used. Therefore, variable reports of polyphenol structure in tea leaves have been published, which, in general, makes the comparison of the results difficult. So, when estimating polyphenol content of tea leaves these factors need to be considered with respect to the reliability of the results.

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