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Improving the Polyphenol Content of Tea

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Tea, prepared from the leaves of *Camellia* species, has one of the highest contents of flavonoids among common food and beverage products. Tea consumption has moved beyond its pleasant flavor and cultural significance since a number of health promoting properties have been ascribed to this widespread beverage (e.g., anticancer, antiobesity and hypotensive effects). The major bioactive compounds in tea are catechins (flavan-3-ols), a group of flavonoids that include, among others, (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epigallocatechin-3-gallate (EGCG). These compounds are also the precursors of theaflavins and thearubigins, oxidation products responsible for the taste and colour of certain tea types such as black tea. The composition of the tea leaf, and thus tea quality, is influenced by many pre-harvest factors such as the genetic make-up of the plant, region of production, horticultural and harvesting practices, and environmental conditions. Once harvested, processing, brewing, and storage conditions influence the phenolic composition and quality of tea infusions as well. In the present review we aim at outlining our current knowledge about means to increase the catechin content of teas, a cornerstone for improving the health-promoting properties of this beverage.

Keywords catechins, flavonoids, plant stress, seasonal variations, theaflavins, thearubigins, pre-harvest, post-harvest, processing

I. INTRODUCTION

Tea is the most popular non-alcoholic beverage in the world (Chen and Zhou, 2005). All kinds of tea originate from *Camellia* species. The main tea types are green, oolong and black, which are essentially determined by the degree of fermentation of its catechins (flavan-3-ols). In addition, there exist some other minor tea types such as white and pu-erh teas. Annual production of tea is estimated at 3.92 million tonnes with black tea and green tea representing the majority of tea production (60% and 30%, respectively) (FAO, 2010). In the future, green tea production, the consumption of which has been largely limited to Japan and China (Sajilata *et al.*, 2008), is expected to increase at a faster rate than that of black tea and other quantitatively minor tea types (FAO, 2010). Green tea is produced in Japan and China from the small-leaf variety *C. sinensis* as it contains less polyphenols than the large-leaf variety, *C. assamica*, used for black tea production (Takeo, 1992; Chu, 1997). The lower flavanol content of *C. sinensis* gives the required astringency to the infusion without being excessively bitter (Takeo, 1992). Black tea is produced in countries in Asia, Africa and Latin America (FAO, 2010). Oolong tea, a semi-fermented tea predominantly manufactured in China and Taiwan, is produced from plants belonging to the variety *C. sinensis*. Clones are selected to impart a strong flowery aroma to this product (Takeo, 1992).

Green tea, and to a lesser extent black tea, has been the focus of many studies demonstrating health-promoting effects of the beverage and its polyphenols, in particular the catechins and their oxidation products, as covered by recent reviews dealing with protection against cancer (Beltz *et al.*, 2006; Yang *et al.*, 2007; González de Mejía *et al.*, 2009), cardiovascular disease (Cheng, 2006; Hodgson and Croft, 2010; Bahorun *et al.*, 2012), diabetes, obesity (Kao *et al.*, 2006; Wolfram *et al.*, 2006; Rains *et al.*, 2011; Uchiyama *et al.*, 2011) and metabolic syndrome (Thielecke and Boschmann, 2009). As evidenced by the investigations summarized in these reviews the flavanol, (-)-epigallocatechin-3-*O*-gallate (EGCG) (Figure 1), is the major bioactive tea polyphenol demonstrating diverse pharmacological activities including antioxidant activity, which is believed to be an underlying protective mechanism in many disease conditions. As a result, the use of tea has moved beyond a beverage enjoyed for its pleasant aroma and taste. Green tea extract and its polyphenols have found application as therapeutic agents and functional ingredients in foods (Wang *et al.*, 2000a; Sajilata *et al.*, 2008; Perumalla and Hettiarachchy, 2011; Zhang *et al.*, 2012). Theaflavins, flavanol oxidation products present in black tea, have comparable antioxidant activity to the major flavanols (Leung *et al.*, 2001), and although not investigated to the same extent as green tea flavanols, these compounds are also increasingly the focus of studies demonstrating health-promoting properties as reviewed by Sharma and Roa (2009). While black tea extracts are mainly used for ready-to-drink tea products, potential new applications, such as prevention of diet-induced obesity (Uchiyama *et al.*, 2011) are highly relevant in the context of the growing prevalence of obesity in developed countries.

The tea plant, originating from mainland south-east Asia, is widely cultivated in this region (China, India, Taiwan, Sri Lanka, and Indonesia), Japan and in central African countries. The plants were traditionally propagated and bred through seeds, but this practice led to genetic variability, loss of yield consistency, and poorer beverage quality (Banerjee, 1992). Therefore, vegetative propagation has become a widespread practice (Banerjee, 1992). New clones of tea are generally selected based on criteria such as beverage quality, yield, ease of establishment, pest resistance, and frost resistance. Currently, there are over 600

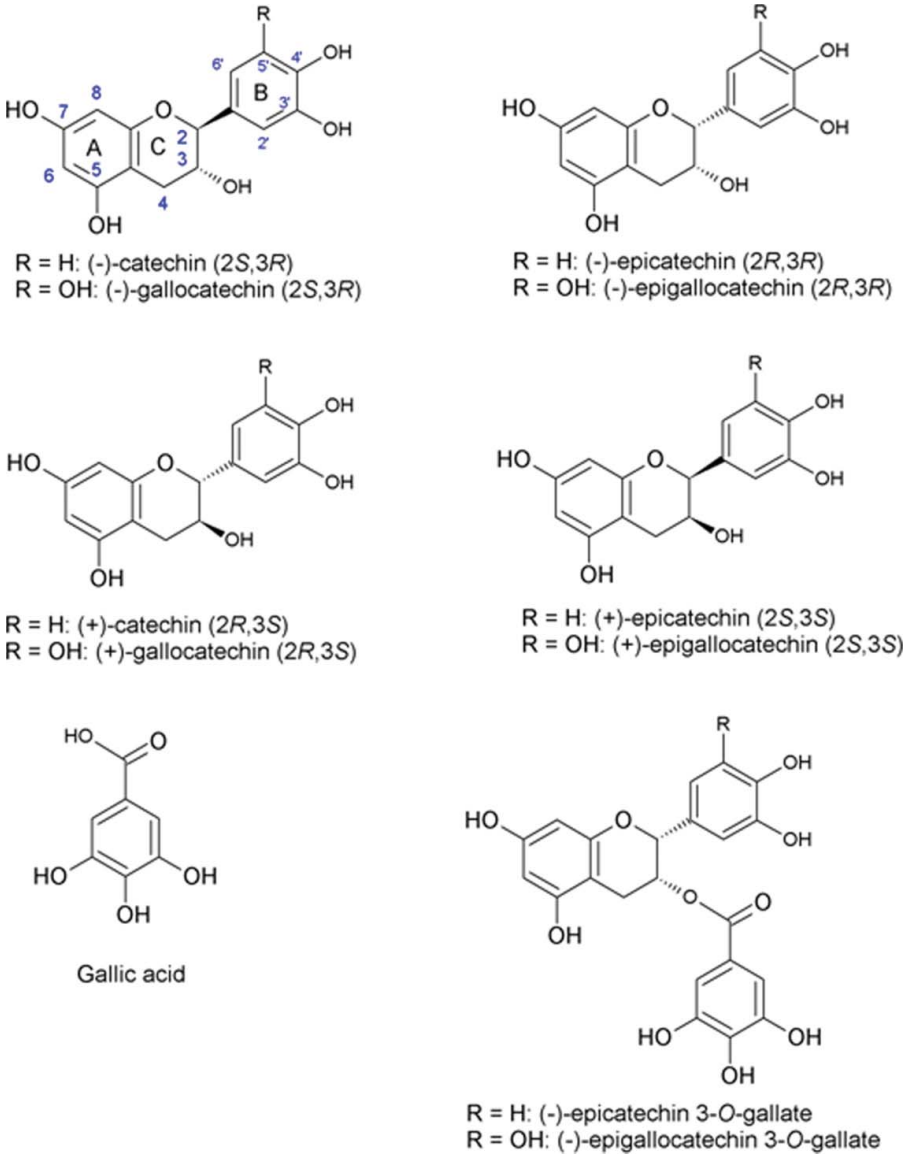


FIG. 1. Chemical structure of the most important groups of tea catechins and catechin-related polyphenols (color figure available online).

cultivated tea varieties, of which many have unique traits such as high caffeine content, blister blight disease tolerance, etc. (Mondal *et al.*, 2004).

Tea has one of the highest flavonoid contents among common food and beverage products (over 10%, USDA 2003). Due to the sensory and health-promoting properties of polyphenols, these compounds are considered important markers of tea quality. It is well known that tea composition, including the levels of polyphenols, and therefore tea quality, is influenced by many factors such as the genetic background of the plant, region and altitude where grown, climatic conditions, horticultural practices, plant nutritional status, plant and leaf age (position of the leaf on the harvested shoot), harvest season, etc. (Owuor and Othieno, 1988; Owuor *et al.*, 1990, 2000, 2008, 2010; Owuor and Odhiambo, 1994; Magoma *et al.*, 2000; Yuan *et al.*, 2000; Owuor, 2001; Han *et al.*, 2002). Most of these factors affecting tea polyphenol content, and thus tea quality, are susceptible to significant improvement (Owuor and Obanda, 1996; Gulati *et al.*, 2009; Sabhapondit *et al.*, 2012). The aim of this work is to outline our current understanding of ways to enhance polyphenol contents in tea plants, both through non-transgenic and transgenic approaches. However, since tea plants have been proven difficult to transform with the currently available techniques (Mondal *et al.*, 2001, 2004; Sandal *et al.*, 2007), we will pay special attention to non-transgenic means to improve the polyphenol levels in the tea leaves. The role of processing, storage and brewing of tea on its phenolic composition, with the emphasis on the flavanols and their oxidation products will also

be discussed as these factors influence the phenolic composition of the product consumed.

II. TAXONOMY AND MORPHOLOGY

In this manuscript we will use the taxonomy described by Sealy (1958) and modified by Wight (1962). According to the latter, all tea plants derive from two *Camellia* species: *C. sinensis* (L.) O. Kuntze (China tea or Small Leaf Tea) and *C. assamica* (Masters) H.T. Chang (Assam tea). According to this classification Cambod tea is a variety of *C. assamica*, *C. assamica* var. *lasiocalyx* (Planchon ex Watt), which shows intermediate features between *C. sinensis* and *C. assamica* (Wight, 1962). It has to be noted, though, that this classification is still a matter of debate, and some authors (for instance, Kaundun *et al.*, 2000) claim that all tea plants come from the same species, *Camellia sinensis* (L.) O. Kuntze, *C. sinensis* var. *sinensis* being the China tea, and *C. sinensis* var. *assamica*, Assam tea. According to this classification the taxonomical level of Cambod tea is, however, unclear. Moreover, *Camellia* taxa hybridise naturally and show a high degree of allogamy (Banerjee, 1992; Gulati *et al.*, 2009). It is generally agreed that at least those three taxa, and to some extent *C. irrawadiensis,* have contributed to the genetic pool of tea (Banerjee, 1992). Furthermore, owing to this extensive hybridization, several intergrades, introgressants and putative hybrids have been formed (Mondal, 2002). The term 'tea,' therefore, covers progenies of the above-mentioned taxa and their hybrids. Despite the complex origin of the genetic pool of tea plants, the numerous hybrids currently available are still referred to as China, Assam or Cambod tea depending on morphological proximity to the main taxon (Banerjee, 1992).

The tea plant is an evergreen tree that blooms in spring and grows up to 9 m high, although cultivated tea plants are kept at waist height by regular plucking. Tea plants show lanceolated or elliptic leaves 2–3 cm wide that vary in size length depending on the species and variety: China tea plants have leaves 5–12 cm long, Assam tea plants have leaves 15–20 cm long and Cambod tea has intermediate-length leaves. Young leaves are pubescent when they sprout and become hairless when mature. The tea plant is naturally from mainland south-east Asia and requires shade and well-drained, slightly acidic, soil for optimum growth (Squire and Callander, 1981).

III. COMPOSITION OF TEA LEAVES

The major constituents of fresh tea leaves are the polyphenols, constituting up to 30% of the dry weight (DW) of young shoots (Robertson, 1992). Literature mostly deals with the phenolic content of processed tea, but insight can be obtained studying the phenolic composition of green and white teas as these tea types suffer little processing beyond the heat inactivation of the endogenous enzymes responsible for oxidation of the polyphenols. Exposure to heat during manufacture may, however, result in the formation of new compounds due to epimerization (Robertson, 1992; Drynan *et al.*, 2010). Zhao *et al.* (2011) recently reported quantitative data for catechins, flavonols, flavones, proanthocyanidins and phenolic acids, present in green and white teas. Of these the catechins (Figure 1), and specifically EGCG, (-)-epigallocatechin (EGC), (-)-epicatechin-3-*O*-gallate (ECG), and (-)-epicatechin (EC) are the most abundant (Wang *et al.*, 2000b; Friedman *et al.*, 2005, 2009a) with the leaf bud and first leaves being the richest in EGCG (Saijo *et al.*, 2004). Studies on the catechin content of fresh leaf of Taiwan varieties showed large variation (Table 1) with all of the above catechins, except with some catechins not detected in some samples due to age of the leaf, plucking season or variety. Other tea catechins present in smaller quantities in the leaf include (-)-gallocatechin (GC), (+)-catechin and *O*-methylated derivatives of EGCG (Chiu and Lin, 2005; Friedman *et al.*, 2009a; Zhao *et al.*, 2011), with young leaves containing higher levels of the *O*-methylated derivatives (Chiu and Lin, 2005). The catechins are not evenly distributed throughout the leaf structure, but occur mainly in mesophyll cells in close proximity to epidermal cells. The catechins are localizsed in large vacuoles within the cells (Suzuki *et al.*, 2003).

Proanthocyanidins, which co-exist with monomeric catechins in many plants, are present in low quantities in the tea leaf (Lakenbrink *et al.*, 1999; Tanaka *et al.*, 2010; Fraser *et al.*, 2012). Several B-type proanthocyanidin dimers have been isolated from fresh tea leaf (Kumar *et al.*, 2009). Procyanidin

TABLE 1
Concentration range of individual catechins in fresh tea leaf (mg g^{-1} DW) of Taiwan varieties

Plant material	Extraction procedure	C	EC	ECG	EGC	EGCG
Different plucking positions[a]	1% (w v^{-1}), boiling water, 30 min	nd – 1.15	nd – 3.76	nd – 1.33	nd – 21.55	0.25 – 8.10
Different plucking positions[b]	2% (w v^{-1}), boiling water, 10 min	3.0 – 6.5	0.06 – 2.4	nd – 5.7	2.3 – 10.3	8.0 – 29.4
Different plucking positions[c]	1% (w v^{-1}), water at 90°C, 3 min	nd – 0.2	nd – 0.7	nd – 1.0	0.1 – 2.9	0.2 – 4.5
Tea varieties (n = 18)[c]	1% (w v^{-1}), water at 90°C, 3 min	nd – 0.2	0.1 – 1.2	0.2 – 0.7	0.5 – 4.6	1.0 – 6.6
Different harvest times[c]	1% (w v^{-1}), water at 90°C, 3 min	0.1 – 0.4	1.0 – 2.1	0.8 – 1.4	4.2 – 12.5	6.4 – 10.6

Values are given as mg g^{-1} DW.

Adapted from [a] Lin *et al.* (2003), [b]Chen *et al.* (2003), [c]Chiu and Lin (2005); nd – not detected. C, Catechin; EC, Epicatechin; ECG, Epicatechin gallate; EGC, Epigallocatechin; EGCG, Epigallocatechin gallate.

and prodelphinidin oligomers, as well as mixed type oligomers, are present in green tea (Kalili and de Villiers, 2010). Various flavonols (up to 4%), flavones (in traces) and phenolic acids are present in fresh tea leaves. The major flavonols in tea are conjugates of quercetin and kaempferol with the conjugating moiety varying from mono- to di- and triglycosides with the associated sugar moieties being most often glucose or rhamnose (Del Río *et al.*, 2004; Zhao *et al.*, 2011). The flavones are mostly *C*-glycosides of apigenin (Drynan *et al.*, 2010). Phenolic acids comprise largely of quinic acid esters of gallic acid and caffeic acid (Del Río *et al.*, 2004; Zhao *et al.*, 2011). The biological significance of these compounds was recently reviewed by Han *et al.* (2007), Khadem and Marles (2010) and De la Iglesia *et al.* (2010). Flavonol glycosides are the main contributors to the astringency of black tea infusions (Scharbert *et al.*, 2004a).

Other compounds of interest in tea leaves include theanine (also called γ-glutamylethylamide or 5-N-ethyl-glutamine) and the methylxanthine caffeine, both found at about 1% in dry tea leaves (Cimpoiu *et al.*, 2010; Ying *et al.*, 2012). Theanine accounts for more than 60% of the total amino acids and is largely responsible for the brothy ("unami") taste of green tea (Takeo, 1992; Juneja *et al.*, 1999). It produces a noticeable relaxation effect in humans (Juneja *et al.*, 1999). Caffeine, a well-known stimulant with several pharmacological properties (Heckman *et al.*, 2010), contributes to the bitter taste of tea and interacts with tea polyphenols to form "tea cream" (Charlton *et al.*, 2000; Couzinet-Mossion *et al.*, 2010).

IV. BIOSYNTHESIS OF FLAVANOLS

Isotope tracer experiments showed that catechins are synthesized in higher plants via the shikimate pathway (Das and Griffiths, 1967; Iwasa, 1977). This pathway leads to the formation of chorismate, which is the precursor of the aromatic amino acids phenylalanine, tyrosine and tryptophan. Phenylalanine (and in some cases tyrosine, but not in the case of tea plants) is the primary precursor of catechins (Zaprometov and Nikolaeva, 2003; Figure 2). In bacteria, chorismate is sequentially transformed into prephenate, arogenate, and phenylalanine by the action of chorismate mutase, prephenate aminotransferase and prephenate dehydratase, respectively (Herrmann, 1995; Zhang *et al.*, 1998; Cho *et al.*, 2007; Yamada *et al.*, 2008). In contrast to microorganisms, the metabolic route from chorismate to phenylalanine in plants is still not entirely known. Even though arogenate has been reported to be a precursor for phenylalanine in plants (De-Eknamkul and Ellis, 1988; Siehl and Conn, 1988), an enzyme converting prephenate into arogenate has not been identified yet. Recent studies showed that *Arabidopsis* plants possess a functional metabolic route from prephenate via phenylpyruvate into phenylalanine, but it is unknown whether this is the case in other plant species (Tzin *et al.*, 2009). For the biosynthesis of flavonoids, phenylalanine is deaminated by phenylalanine ammonia lyase (PAL) giving rise to cinnamic acid, which is sequentially transformed into coumarate and 4-coumaroyl-CoA (Figure 2). The first committed step in the biosynthesis of all flavonoids, including catechins, is the condensation of 3 malonyl-CoA molecules with 4-coumaroyl-CoA to yield a chalcone in a reaction catalyzed by chalcone synthase, a polyketide synthase (reviewed by Petit *et al.*, 2007; Wang *et al.*, 2011). Chalcone isomerase catalyzes the stereospecific isomerization of chalcones to the corresponding flavanones (Moustafa and Wong, 1967). From these intermediates, the pathway diverges into several side branches, each resulting in a different class of flavonoids. Flavanone 3-hydroxylase catalyzes stereospecific 3-hydroxylation of (2*S*)-flavanones to the (2*R*, 3*R*)-3-hydroxyflavanones (dihydroflavonols; for instance naringenin into dihydrokaempferol) (Forkmann *et al.*, 1980). Dihydroflavonols (e.g., dihydrokaempferol, dihydroquercetin and dihydromyricetin) are reduced to leucoanthocyanidins (e.g., leucopelargonidin, leucocyanidin and leucodelphinidin) by dihydroflavonol 4-reductase, a NADPH-dependent enzyme (Reddy *et al.*, 1987). Flavanones and dihydroflavonols may be further hydroxylated, either at the 3′ position or at both 3′ and 5′ positions of the B-ring by the P450 hydroxylases, flavonoid 3′-hydroxylase (F3′H) and flavonoid 3′5′-hydroxylases (F3′5′H), respectively (Toda *et al.*, 2002; Ueyama *et al.*, 2002; Chopra *et al.*, 2006). Both enzymes require oxygen and NADPH as co-factor.

As shown in Figure 1, catechins have 2 chiral centres at carbons 2 and 3, thus there are 4 possible isomers for each molecular formula. For instance, (-)-catechin (2*S*,3*R*-catechin), (+)-catechin (2*R*,3*S*-catechin), (-)-epicatechin (2*R*,3*R*-catechin), and (+)-epicatechin (2*S*,3*S*-catechin). The action of F3′H and F3′5′H on the hydroxylation pattern of the C ring (Figure 2) allows the formation of three sets of catechins: afzelechin-type catechins (flavan-3-ols with the C ring hydroxylated only at C4′), catechin-type catechins (flavan-3-ols with the C ring hydroxylated at C3′and C4′) and gallocatechin-type catechins (flavan-3-ols with the C ring hydroxylated at C3′, C4′ and C5′) (Figure 2). For the biosynthesis of the *cis* catechins (e.g. (+)-epiafzelechin, (-)-epiafzelechin (+)-epicatechin, (-)-epicatechin, (+)-epigallocatechin and (-)-epigallocatechin), the achiral leucoanthocyanidins are converted into anthocyanidins in a reaction catalyzed by anthocyanidin synthase via hydroxylation at C3 to give an intermediate which spontaneously isomerizes to a thermodynamically stable achiral anthocyanidin pseudobase (Heller and Forkmann, 1994). Anthocyanidin reductase yields the mentioned *cis* flavan-3-ols from anthocyanidins (Gargouri *et al.*, 2009). In contrast, for the biosynthesis of the *trans* epimers (e.g., (+)-afzelechin, (-)-afzelechin, (+)-catechin, (-)-catechin, (+)-gallocatechin and (-)-gallocatechin), the achiral leucoanthocyanidins are transformed into the mentioned *trans* flavan-3-ols by the action of leucoanthocyanidin reductase (reviewed by Heller and Forkmann, 1994; Gargouri *et al.*, 2009; Wang *et al.*, 2011, Figure 2). Catechins can undergo esterification with gallic acid to form catechin gallates, and hydroxylation reactions to form gallocatechins such as EGC, EGCG, and ECG (Friedman *et al.*, 2005).

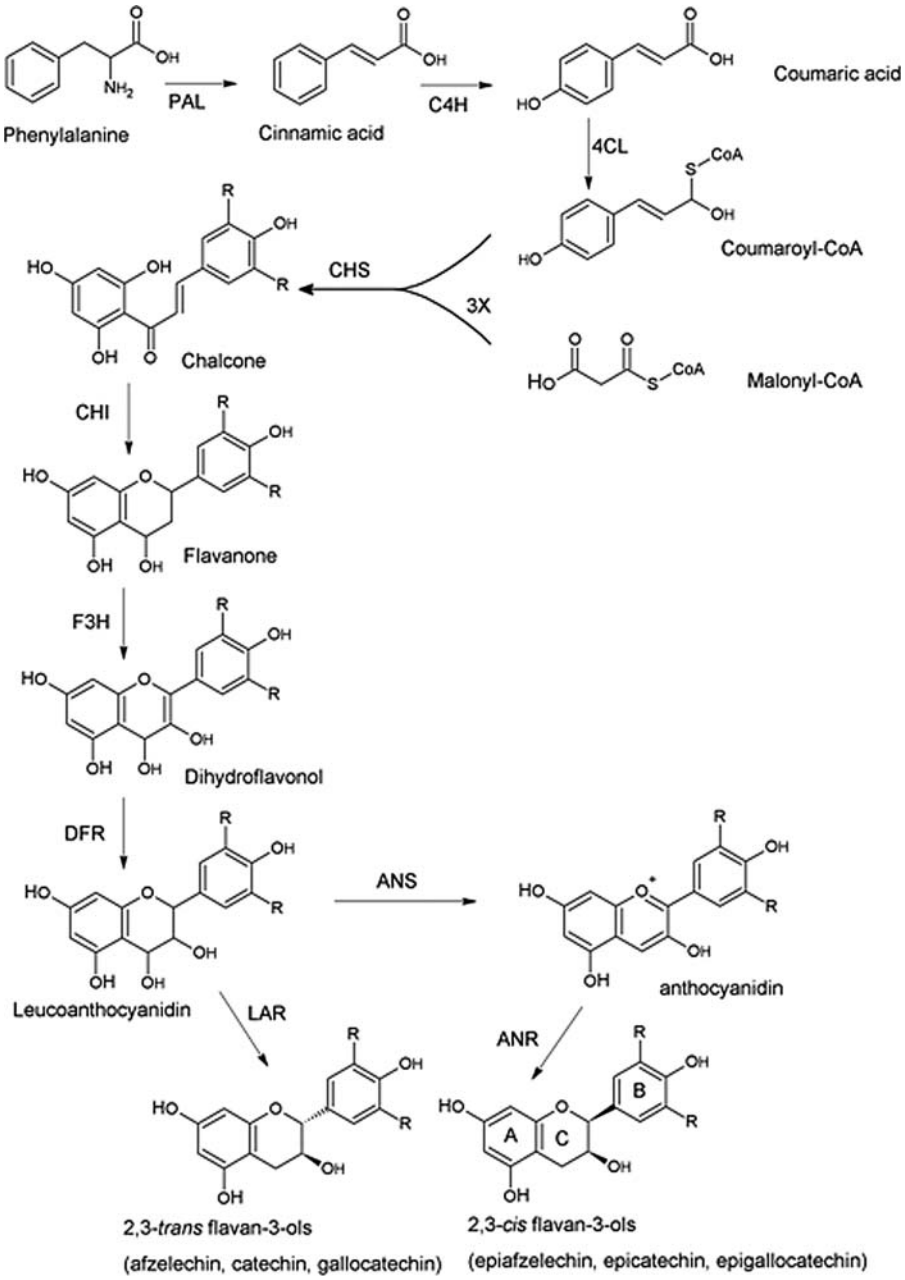


FIG. 2. Outline of the flavan-3-ol biosynthetic pathway in tea plants. PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumaroyl CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; DFR, dihydroflavonol reductase; ANS, anthocyanidin synthase (syn. leucoanthocyanidin dioxygenase); LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase. F3′H and F3′,5′H (flavonoid 3′ hydroxylase and flavonoid 3′,5′ hydroxylase, respectively) catalyze the hydroxylation of the B ring of flavanones and dihydroflavonols, yielding flavonoids with different B ring hydroxylation patterns (for instance, dihydrokaempferol, dihydroquercetin and dihydromiricetin are dihydroflavonols mono-, di-, and tri-hydroxylated in their B rings).

V. GENOTYPE SELECTION TOWARDS HIGH FLAVANOL CONTENT

As a beverage crop with worldwide significance, extensive tea germplasm collections have been set up in China, India and other countries (Wachira *et al.*, 2001). As mentioned before, and according to Wight's classification, the cultivated tea taxa include two species: *C. sinensis* (L.) O. Kuntze or China type and *C. assamica* (Masters) H. T. Chang, which includes Assam type tea and Cambod or Southern tea (*C. sinensis* var *lasiocalyx* (Planchon ex Watt), Wight, 1962). However, tea is very heterogeneous (Gulati *et al.*, 2009), and all the above and many other taxa freely interbreed, and the resulting lines are

arranged based on morphological characters that extend from China types through intermediates to those of Assam types (Wight, 1962). Hybridization has been so common that it is often debated whether *C. sinensis* and *C. assamica* archetypes still exist (Bezbaruah, 1976). Currently, over 600 varieties cultivated world-wide are available, and they are still referred to as China, Assam or Cambod tea depending on morphological nearness to the main taxon (Banerjee, 1992). Particularly, two interesting wild taxa, i.e., *C. irrawadiensis* and *C. taliensis*, which morphological distributions overlap with that of *C. sinensis*, have been shown to contribute considerably to the genetic pool of tea (Banerjee, 1992). Other *Camellia* species, which are assumed to have contributed to the tea genetic pool by hybridization, include *C. flava* (Pifard) Sealy, *C. petelotii* (Merrill) Sealy and possibly *C. lutescens* Dyer (Wight, 1962; Sharma and Venkataramani, 1974). Widespread cultivation of clonal tea for high yield and uniform quality may lessen the genetic diversity, so conservation of germplasm resources is central for sustainability of the tea industry (Sabhapondit *et al.*, 2012).

Since the levels and proportion of tea catechins are under genetic control (Gerats and Martin, 1992), it has been suggested that the levels of total catechin and/or some individual catechins might be used also as markers to differentiate between the three major varieties (*C. sinensis*, *C. assamica*, and *C. assamica ssp. lasiocalyx*). Indeed, the biochemical fingerprinting of catechins has been successfully applied in a number of studies. For instance, Takeda (1994) showed that among 1500 tea cultivars, Japanese cultivars were much more homogeneous than other cultivars in terms of tannin and caffeine content. Given that a higher hydroxylation pattern usually confers higher antioxidant capacity to flavonoids, the total green leaf catechin content and the ratio of dihydroxy (EC + ECG) to trihydroxy (EGC + EGCG) catechins were used to establish genetic diversity in the tea germplasms of Kenya (Magoma *et al.*, 2000). Similarly, biochemical fingerprinting of the tea varieties based on catechin composition in green leaf of cultivars (including China, Assam, and Cambod varieties) grown in the Northeast of India has been also reported with the results showing large variations in individual catechins and total catechins among the cultivars that reflected the genetic variability (Sabhpondit *et al.*, 2012). As a general rule, Assam cultivars contain the highest catechin contents (231 mg g^{-1}DW) followed by Cambod (202 mg g^{-1}DW), and China cultivars (157 mg g^{-1}DW) (Sabhapondit *et al.*, 2012). Similar results were obtained by Sharma *et al.* (2011), who also found that the second largest contributor to total catechin content was EGC for Assam variety, while it was ECG for Cambod and China varieties (the first one is EGCG in all three varieties). From this study one can conclude that a high EGC content is characteristic of the Assam variety. In a similar manner, many other studies have reported on biochemical particularities of tea varieties from China, Himalaya, and India (Chen and Zhou, 2005; Karthigeyan *et al.*, 2008; Sabhapondit *et al.*, 2012).

Altogether these variations indicate that selection for high quality tea requires accurate information on the chemical diversity and biochemical characterisation of such diversity, and that this diversity seems a useful tool in the development of quality tea clones (Gulati *et al.*, 2009). However, the same studies show that selection of a genotype, based solely on catechin content, does not guarantee the production of a tea with 100% predictable catechin composition. Other factors, as described below, also affect tea catechin content.

VI. EFFECTS OF GEOGRAPHICAL ORIGIN AND CLIMATIC FACTORS

The tea plant is grown in countries ranging from as far north as 49°N (outer Carpathians, Russia) to as far south as 30°S (Natal, South Africa) (Shoubo, 1989; Owuor *et al.*, 2010). The tea plant is widely adaptable to geographical areas with large variations in climate and physical features, which affect the growth rates, yields and quality (Owuor *et al.*, 1986; Owuor and Obanda, 1996). In order to minimize the variability caused by the growing conditions, many countries have placed their tea breeding/improving centers in single locations (Owuor *et al.*, 2010), but tea plants selected in one location and planted in another usually do not match the performance shown at the site of selection (Wachira *et al.*, 2002). Several studies have demonstrated wide response ranges of tea plants to changing growing environments including soil types, soil fertility, temperatures, water stress, rainfall distribution, and growing altitude (Tanton, 1982; Obaga *et al.*, 1989; Bonheure and Willson, 1992; Othieno *et al.*, 1992; Squire *et al.*, 1993; Balasuriya, 1999; Ng'etich *et al.*, 2001; Wachira *et al.*, 2002). The variation in the total and individual catechin contents of tea evaluated among three different cultivars showed that the variation in catechin contents among locations were far larger than those among cultivars, indicating that environmental effects should be taken into consideration when total and individual catechins are utilized as biochemical markers in a tea breeding program (Table 2). Wei *et al.* (2011) reported that (+)-catechin was the catechin most affected by weather, and that its levels were negatively correlated with daily lowest temperature, but positively correlated with precipitation and sunlight exposure time. Shifts in (+)-catechin content might alter the organoleptic properties of tea, since (+)-catechin is notably less bitter and astringent than other catechins (Kallithraka *et al.*, 1997). Although other catechins are far more abundant (for instance, (+)-catechin content is about one third of that of EGCG), (+)-catechin should not be ignored in the evaluation of green tea quality (Wei *et al.*, 2011). These authors also suggested that climate factors, mainly daily lowest temperature and sunlight exposure time, do not directly influence the rate of biosynthesis of (+)-catechin, but regulate its content through the formation of chlorophyll. Indeed, it appears that the high negative correlation between chlorophyll and (+)-catechin content found in three Chinese tea cultivars (Wuniuzao, Longjing 43, and Fudingdabaicha) might be closely associated with leucocyanidin reductase (LAR) and anthocyanidin synthase (ANS) activities (Skadhauge *et al.*, 1997; Abrahams *et al.*, 2002). LAR

TABLE 2
Levels of the major tea catechins under different environmental conditions

	Water stress			High altitude		Season	
	Control	Water deficit	Water logging	350 m	500 m	Spring	Autumn
(-)-Epicatechin	1.24 ± 0.09^{a}	1.10 ± 0.20^{a}	1.20 ± 0.3^{a}	4.7 ± 0.6^{b}	5.5 ± 0.6^{b}	4.2 ± 0.6^{b}	5.5 ± 0.6^{b}
(-)-Epicatechin gallate	2.62 ± 0.58^{a}	1.20 ± 0.20^{a}	2.40 ± 0.30^{a}	16.0 ± 0.1^{b}	17.6 ± 1.9^{b}	16.8 ± 2.0^{b}	17.6 ± 1.9^{b}
(-)-Epigallocatechin	3.18 ± 0.14^{a}	3.80 ± 0.90^{a}	3.80 ± 0.30^{a}	25.6 ± 1.0^{b}	25.2 ± 1.3^{b}	20.8 ± 3.1^{b}	25.2 ± 1.3^{b}
(-)-Epigallocatechin gallate	28.79 ± 2.89^{a}	12.41 ± 2.51^{a}	24.92 ± 0.83^{a}	90.6 ± 5.5^{b}	103.4 ± 3.1^{b}	102.8 ± 0.7^{b}	103.4 ± 3.1^{b}

Values are given as mg g^{-1} DW.
Adapted from [a]Jeyaramraja *et al.* (2003) (3 cultivars) and [b]Chen *et al.* (2010) (1 cultivar).

is the only enzyme characterized to catalyze the conversion of leucocyanidin to (+)-catechin in several plants (Abrahams *et al.*, 2002), and its activity commonly decreased during tissue development (Skadhauge *et al.* 1997). However, ANS is a 2-oxoglutarate iron-dependent oxygenase, which uses molecular oxygen as the co-substrate (Cheng *et al.*, 2007). Consequently, the formation of chlorophylls will increase plant photosynthesis and then lead to sink-source alterations mainly in young leaves. The increase in chlorophyll *a* content during young leaf development correlated with the increase of EC, EGC and the decrease of (+)-catechin for both Wuniuzao and Longjing 43 tea cultivars, which might be due to reduction of LAR activity and enhancement of ANS activity.

It is therefore clear that climatic conditions are of great importance for the composition of tea leaves and thus, have a great impact on the quality of downstream tea products. Furthermore, several reports have shown that minor changes in the climate can provoke significant changes in catechin levels in tea plants (Hazarika *et al.*, 1984; Mahanta *et al.*, 1988). In the Kangra valley (India), the early growth flush coincides with dry weather, cooler nights and desiccating winds that favour the biogenesis of aroma compounds. A strong positive correlation was observed between total catechins and prephenate dehydratase activity for both Assam and China varieties in three growth flushes within this valley (Sharma *et al.*, 2011).

A. Effect of Growth Altitude on Tea Catechin Content

A factor that deserves special mention is the growth altitude (Table 2). Tea plants are cultivated at altitudes varying from sea level in Japan to 2700 m above mean sea level (amsl) in Olenguruone (Kenya) and Gisovu (Rwanda) (Owuor *et al.*, 2008). When comparing the quality of black tea from Malawi and Kenya, it was shown that even when the same tea clones were used to manufacture black tea, quality variations persisted. The observed variations may be in part due to the non-uniformity of agronomical practices and variable climatic conditions, but the tea from Malawi and Kenya was grown at 650 m amsl, latitude 16°5′S, and 2180 m amsl, 0°22'S, respectively. In Kenya, for example, yields decreased (Ng'etich *et al.*, 2001; Squire *et al.*, 1993) and quality of clonal tea improved (Owuor *et al.*, 1990) with a rise in altitude. The variation in yield was attributed to growth rate and shoot density differences (Obaga *et al.*, 1989; Ng'etich and Stephens, 2001). For every 100 m rise in altitude, there is an estimated loss of 1 kg made tea ha^{-1} (Othieno *et al.*, 1992). In contrast, tea quality (referred to as flavonoid content and composition) improves with slow growth at high altitude (Mahanta *et al.*, 1988; Owuor *et al.*, 1990; Squire *et al.*, 1993; Ng'etich *et al.*, 2001). From these results it was assumed that the changes in yield and quality will change with altitude in an expected manner, though not necessarily at the same rates for different cultivars. All these results support the need to test genotypes in new areas of production to fully evaluate their relative quality potentials. Still, there are tea genotypes that are more resistant to yield variations with location of production and vice versa (Wachira *et al.*, 2002).

B. Taking Advantage of Seasonal Variations of Polyphenol Contents

Although tea plants are cultivated over a wide range of geographical locations, all these locations need to be relatively humid (1000–1400 mm per year) with mild temperatures (USDA hardiness zone higher than 6). All tea producing areas thus fall within A and C climate groups according to the Köppen-Geiger climate classification (tropical and temperate climates), which shows at least two, and usually four different seasons (Köppen. 1900; Geiger and Wolfgang, 1954; Shoubo, 1989). The phenolic levels and composition of tea shoots vary considerably among these seasons. The factors that induce seasonal variations on total phenolic content in tea shoots are mainly day length, rainfall, sunlight, and/or temperature, which vary markedly across seasons (Hilton and Palmer-Jones, 1973). In a recent study, Erturk *et al.* (2010) studied the seasonal variations of total phenolic content and antioxidant activity in fresh tea leaves harvested from different clones cultivated in Turkey during three harvest seasons of two consecutive years. For both years, the total phenolic content of all clones were lower in cool months (May; average 33.00–90.27 mg GAE g^{-1} DW), whereafter their level increased during the warmer months, from July to September. The authors suggested that these differences may be a consequence of changing temperature, day length, and/or irradiance. Nevertheless, the results showed

the potential to produce higher quality tea during September in Turkey. Harbowy and Balentine (1997), in a review of the chemistry of phenolic compounds in tea shoots, concluded that their biosynthesis is induced by stronger sunlight and longer day length, which is in agreement with previous studies reporting that polyphenol content of tea leaves increases with sun exposure (Mahanta and Baruah, 1992). Zheng *et al.* (2008) demonstrated that short-term irradiation with UV-B stimulated accumulation of tea catechins, while excessive exposure to UV-B irradiation supressed their accumulation. Sanderson (1972) reported that a rise in temperature may enhance the biosynthesis of catechins. Tea shoots collected during three growth flushes of two consecutive years, showed large changes in total catechin levels among growth flushes (Sharma *et al.*, 2011). These changes were ascribed to the ambient temperature, the sunlight hours, and/or the rainfall distribution. Higher catechin content was recorded during the main flush when the solar irradiation (in particular UV-B irradiation) and average day temperatures are the highest, followed by early (March-May) and backend (September-November) flushes for both Assam and China varieties. Similar trends were observed for the individual catechins, i.e., EGC, EGCG and ECG. A high positive correlation was observed between the increase in temperature and the catechin content of both China and Assam tea varieties.

All these studies indicate that the levels of total and individual catechins show sharp seasonal fluctuations in response to temperature, day length, irradiance, and/or UV doses. However, since tea is to be grown in temperate climates, these factors are difficult to disentangle from each other, but should be taken into account in future attempts to optimize/maximize flavonoid levels in teas.

VII. EFFECTS OF LEAF AGEING AND STRESS

A. Effects of Leaf Canopy Position and Ageing

Several studies have examined the effects of leaf age (and hence, leaf position) on the contents of tea catechins (Jain, 1999; Xu and Chen, 2002; Lin *et al.*, 2003; Saijo *et al.*, 2004; Chiu and Lin, 2005; Yao *et al.*, 2006; Sharma *et al.*, 2011; Wei *et al.*, 2011). The most actively growing tissue of the tea plants, commonly referred to as "two and a bud" (the apical bud and the two uppermost leaves, including the shoot that holds them) are used to manufacture high-quality tea (Jain, 1999). Such practice was established based on studies showing that tea quality declines notably when older leaves are included (Xu and Chen, 2002; Sharma *et al.*, 2011; Wei *et al.*, 2011). The (+)-catechin content of Wuniuzao and Longjing 43 cultivars declined notably from the one-leaf-and-a-bud stage to the three-leaves-and-a-bud stage (Wei *et al.*, 2011). Furthermore, analysis of tea shoots (two and a bud) belonging to both Assam and China varieties showed that the total catechin content of young tea leaves (first leaf and apical bud) was higher compared to mature ones (second, third, and fourth leaves) (Sharma *et al.*, 2011). Besides, higher EGCG and EGC values were recorded for the bud and first leaf and it decreased with the maturity of the leaves (Xu and Chen, 2002; Wei *et al.* 2011). Similarly it was reported that higher catechin levels were recorded in young leaves of Kangra (Indian) tea and Japanese teas in comparison with older leaves (Hahlbrock, 1981; Sharma *et al.*, 2011), while Park *et al.* (2004) demonstrated higher levels of EGC, EGCG, and ECG in South Korean young tea leaves than in mature leaves. Studies by Barman and Saikia (2005) on the allocation of ^{14}C-labelled assimilates in tea showed that high-yielding clones retained lower amount of photosynthates (sugars) in the maintenance leaves (source) and allocated a higher percentage towards the pluckable shoots (sink), indicating that more precursors are available for catechin biosynthesis in young leaves when compared to older ones.

In contrast, analysis of old and young leaves of plants in a Taiwanese tea breeding program showed higher levels of the catechins in the older leaves, as well as higher levels for EGC than EGCG (Lin *et al.*, 2003). In another study of tea varieties cultivated in Taiwan, EGCG levels were higher in young leaves (apical bud and two youngest leaves), compared to old leaves (fifth to tenth leaf), while the leaves of seven out of sixteen varieties had a higher EGC content than EGCG (Chiu and Lin, 2005). In a study using the Chinese tea cultivar Zhenong 139, the EGC and ECG levels were higher in mature leaves than in young leaves, while the EGCG and gallocatechin gallate (GCG) levels did not show significant differences between young and mature leaves (Mamati *et al.*, 2006). These different results were possibly due to genetic variation and differences in environment and agricultural practices, or combinations of these factors. Furthermore, Eungwanichayapant and Popluechai (2009) showed that the mRNA levels of the genes encoding for phenylalanine ammonialyase 1, chalcone synthase, dihydroflavonol 4-reductase, leucoanthocyanidin reductase, and flavanone 3-hydroxylase were higher in the young shoots than in the mature leaves. The authors concluded that the accumulation of catechins in tea leaves is regulated by the regulation of structural genes at transcriptional level. In further studies, the expression of these genes might be used as biomarkers in tea breeding programs and genetic engineering to improve tea quality.

B. Effects of Nutrient and Water Supply

N fertilizers are routinely used in tea plantations as they significantly increase the levels of free amino acids, thereby improving yield, as well as green tea quality (Owuor, 2001). Free amino acids are well known as important constituents of green tea and are the major contributor to freshness and mellowness of the infusion (Wang *et al.*, 1988; Mukai *et al.*, 1992) or brothy taste (Takeo, 1992; Juneja *et al.*, 1999). In addition, chlorophyll contents are usually increased by N supply (Krishnapillai and Ediriweera, 1986), which causes an undesirable grassy note in black tea, leading to poor quality (van Lelyveld *et al.*, 1989; 1990). Furthermore, application of N fertilizers has also been found to increase the fatty acid content of leaves, leading to a high level of undesirable aroma notes in black tea (Owuor and Odhiambo, 1994). Although these effects are of great

importance to the tea industry, the focus of this section will fall on phenolic compounds.

Limited supply of nutrients is the key factor restricting productivity and quality of tea, which is mostly cultivated in highly leached and strongly acidic soils (Han *et al.*, 2002). A number of experiments have shown the positive effect of nutrients (N, P, K, Mg, S, and micronutrients) on yield and quality (Tea Research Institute, 1985; Wu and Ruan, 1994; Han *et al.*, 2002; Ruan *et al.*, 2003). It is generally believed that increasing N fertilization impairs black tea quality, which is mainly determined by the levels of theaflavins and thearubigins (Hazarika *et al.*, 1984; McDowell *et al.*, 1995; Owuor, 2001). The results regarding the effect of N fertilization on the concentration of individual catechins and their oxidation derivatives (theaflavins and thearubigins) are ambiguous, though. Hilton *et al.* (1973) showed that N fertilization diminished EGC and EC levels in young shoots, while it either increased or decreased EGCG levels. A recent study showed that the total polyphenol content of black tea is increased by N fertilization (Venkatesan and Ganapathy, 2004), while other studies reported a decrease (Cloughley, 1982; Owuor and Odhiambo, 1994), no change (Owuor *et al.*, 2000) or even an increase (Owuor *et al.*, 1987) in theaflavin and thearubigin concentrations. More research needs to be done in this regard to determine the effect of exogenous N on catechin levels in tea plants, and to be able to predict such effects in different tea varieties.

Young tea shoots contain abundant K (15–25 mg g^{-1} DW). Yield increases with application of K fertilizers have been reported (Malenga and Grice, 1991; Wu and Ruan, 1994; Sharma and Sharma, 1995). Besides, the quality of tea also improved upon application of exogenous K due to the increase of total free amino acid in green tea and theaflavins and thearubigins in black tea (Ruan *et al.*, 1999; Venkatesan and Ganapathy, 2004). Compared to KCl, K_2SO_4 has an advantage of concomitant provision of S. The importance of S nutrition in formation of quality determining constituents (free amino acids, caffeine, and catechins) has been reported (Barbora, 1995; Ali *et al.*, 1997; Ruan *et al.*, 1998). Concentrations of theaflavins and thearubigins and the color of black tea are also improved by S application (Barbora, 1995; Ali *et al.*, 1997). Furthermore, Wu and Ruan (1994) reported that the aroma of tea is improved by S application.

Chloride has functions in photosynthesis, stomatal movements, and osmoregulation and is thus a crucial micronutrient for higher plants (Marschner, 1995). However, excessive chloride accumulation in plant tissues is a major problem restricting crop productivity in saline regions. Since tea plants are generally cultivated in humid tropical and subtropical areas with highly leached soils, thus either chloride deficit or toxicity in tea has rarely been reported. However, tea plants are chloride-sensitive plants (Ruan, 2005), and chloride toxicity may arise under particular conditions such as the use of fertilizers containing chloride, i.e., NH_4Cl. Leaf damage occurs when chloride beyond 200 kg ha^{-1} is applied and becomes more severe with increasing amounts of NH_4Cl. Although improved plant nutrition affects mainly the yield and amino acid accumulation, catechin accumulation also responds to improved mineral nutrition. Thus, this factor, together with the soil pH, which determines the ionic form in which different nutrients are present and hence their availability for the plant, should be kept in mind if the intention is to increase the levels of catechins in tea plants.

The productivity of tea is also affected to a great extent by recurring water deficit (Table 2). The effects of soil moisture stress on morphological and physiological parameters have been reported (Chakraborty *et al.*, 2002). Studies on its impact on the biochemical constituents that determine quality attributes of tea are scarce (Jeyaramraja *et al.*, 2003; Hernández *et al.*, 2006; Sharma *et al.*, 2011). Studies in this regard would be useful to understand the degree to which tea quality deteriorates as a result of water stress, and to determine the constituents sensitive to drought. Jeyaramraja *et al.* (2003) reported on the impact of water stress on the biochemical constituents that determine black tea quality. Pot-grown tea clones, UPASI-2 (Assam cultivar) and UPASI-9 (China cultivar) which are drought-tolerant and UPASI-8 (Cambod cultivar) which is drought-susceptible, were submitted to either drought or waterlogging stress for a ten-day period. PAL activity was highest in the drought-tolerant Assam cultivar UPASI-2, followed by UPASI-8 and UPASI-9, under non-stress conditions. Accordingly, the catechins of interest EC, EGCG and ECG were higher in the drought-tolerant cultivar UPASI-9. Moreover, under soil moisture stress, the PAL activity was reduced in all three clones. The levels of individual catechins tended to increase with mild or moderate drought, although in all cases, except EGC in the most drought-sensitive clone UPASI9, they declined to levels lower than in the non-stressed plants. The lower levels of catechins, in particular EGCG and ECG, important precursors of theaflavin-3,3′-digallate, affect the final tea quality in all kinds of tea preparations. Besides, synthesis of quality constituents such as gallic acid and caffeine also decreased significantly due to drought stress. The reduction in gallic acid due to water stress could lead to lower synthesis of theaflavin type compounds such as epitheaflavic acid, epitheaflavic 3′-gallate and theaflavic acid and, thereby, quality deterioration (Jeyaramraja *et al.*, 2003). Sharma *et al.* (2011) also used tea plants from Assam and China varieties to study the effect of water stress on the leaf catechin contents. Two-year-old tea plants were grown in pots and drought stress was imposed by water withholding. After 8 days of treatment the plants were watered to investigate the effect of stress recovery. Drought stress decreased total and individual catechins (EGC, EGCG, ECG, and EC) from the third day on. After 8 days of water deficit, the total catechins decreased by 24% (from 14.85 to 11.28 mg g^{-1} DW) and by 18% (from 11.40 to 9.32 mg g^{-1} DW) for both Assam and China varieties, respectively. Following rehydration, recovery of total and individual catechins was higher in the China variety than the Assam variety (Sharma *et al.*, 2011). Experiments in field-planted tea support a correlation between drought and quality deterioration (Owuor

and Othieno, 1988). A loss of quality and lower theaflavin content were observed for tea made from plants which had suffered from drought. Therefore, these findings emphasize the importance of proper management practices to prevent drought stress. Irrigation, which is already in use in certain tea fields to maintain the optimal soil moisture level, as well as foliar application of antitranspirants and NK (1% each of urea and muriate of potash), may be used favorably to lessen drought and nutrient stress in tea plantations. Similarly to drought stress, Jeyaramraja *et al.* (2003) demonstrated that flooding stress alters the biochemical constituents necessary for tea quality (Table 2). Substantial reductions in gallic acid levels were recorded under flooding conditions, irrespective of the tea clone.

C. Effects of Biotic Stress

During its lifetime of about 100 years, the tea plant has to overcome different types of stress of both biotic and abiotic nature. The major biotic stress that the tea plant encounters is the attack by insect pests, including caterpillars, leaf rollers, flies, bees, wasps, ants, grasshoppers, crickets, locusts, aphids, scale insects and plant bugs (Sudhakaran *et al.*, 2000). Among the insect pests, *Helopeltis theivora* Wat. (tea bug) is perhaps the most important, as every year it causes massive (10–50%) losses to the tea crop. More than 80% of the tea cultivation area is being affected by nymphs and adults of *H. theivora*, leading to dark brown shrunken spots on young foliage, no shoot formation, delayed flushing, stunted growth and die back of stems. The biochemical response of 12 Indian tea varieties to the attack by *H. theivora* was determined by Chakraborty and Chakraborty (2005), and the results showed that the insect attack was severe during the high temperature and rainfall months, from May to September, which led to severe loss of biomass due to leaf curling and drying. As a response to *H. theivora* damage, the activities of several oxidative enzymes and polyphenol oxidase (PPO) increased, whereas PAL activity decreased (Chakraborty and Chakraborty, 2005). Consequently, decreases in the total polyphenol content with insect attack were found. The most susceptible tea varieties showed the largest reduction in total polyphenol content. Furthermore, the attack by tea bugs reduced both GCG and catechin gallate (CG) content, while slightly increased EC content (Chakraborty and Chakraborty, 2005). However, in the same study it was shown that the theaflavin content of black tea manufactured from damaged leaves compared to tea manufactured from healthy leaves was not affected, which may be due to the increased activity of PPO during insect damage. It was concluded that insect attack did not affect the tea flavor component, but was responsible only for a strong reduction in biomass (Chakraborty and Chakraborty, 2005).

The foliar disease, blister blight, caused by the obligate parasitic fungus *Exobasidium vexans* Massee, is considered as one of the economically important disease that hinders tea production (Muraleedharan and Chen, 1997). For instance, in Sri Lanka, the tea plants lost approximately 33% of their yield due to blister blight in unprotected areas compared to fields which were sprayed with chemicals (de Silva *et al.* 1977). Tea shoots (two leaves and a bud) showed qualitative and quantitative variations in total catechins as affected by blister blight. Besides, the *Exobasidium vexans* Massee fungus infects only the tender leaves and stem, with the severely affected shoots producing tea of very poor quality (Baby *et al.*, 2000). Plants affected by this fungus showed decreases in polyphenol and catechin levels (Premkumar *et al.*, 2008; Sharma *et al.*, 2011). Such decreases were more pronounced in tea shoots that were 50% or more infested by blister blight. The loss in the quality marker consituents could be due to extensive fungal damage to the young succulent tissue, i.e., mainly the palisade and the epidermal layers containing the stomata resulting in lower photosynthetic rates and thereby indirectly reducing the carbon flow through the shikimate pathway (Sharma *et al.*, 2011). The reduction in tea polyphenols and catechins may also be attributed to the secretion of certain metabolites that degrade them or to their utilization by the pathogen (Premkumar *et al.*, 2008). These authors also showed a depletion of sugars in blister blight diseased leaves, which may affect the biosynthesis of polyphenols as sugars are their precursors. On the other hand, there are reports showing an increase in the flavonoid content of other plants such as *Pteridium aquilinum* Kuhn and *Glycine max* after fungal infection and herbivore damage (Tempel, 1981; Chiang and Norris, 1983).

It thus appeared that insect attack strongly reduced the plant biomass as determined in 12 tea varieties, while their quality components were not significantly affected. The theaflavins were not affected in black tea manufactured from damaged leaves as compared to healthy leaves. However, if some of the phenolic constituents decreased, these decreases were more significant in the most susceptible tea varieties. One of the reasons for the observed tolerance of certain varieties to insect attack could be their ability to maintain higher levels of phenolics in the face of attack. Still, to overcome both insect attack and the hazardous effect of insecticides, it is important to consider the new bio-rational insecticide derived from natural sources, i.e., plant extracts, insect pathogens, etc., which is a universally acceptable and practicable approach worldwide since it is relatively innocuous to nontarget organisms. It appeared that fungal diseases such as *Exobasidium vexans* Massee were harsher on both tea yield and quality. Moreover, others fungus such as *Macrophoma theicola* can kill the entire plant through twig dieback and stem canker (Keith *et al.*, 2006).

VIII. GENETIC AND MOLECULAR BIOLOGY APPROACHES TO IMPROVE POLYPHENOL LEVELS IN TEA

Since the world market has critical standards for tea produced in different parts of the world, crop improvement combining both yield and quality is essential (Jain, 1999). However, tea has several constraints for its improvement. Seed-grown tea is heterogeneous due to their highly allogamous nature; therefore, it is difficult to preserve their higher character. Besides,

vegetative propagation is yet limited by slow multiplication rate, poor survival of some clones, and the need for plentiful initial planting material. Conventional tea breeding is well established, though time-consuming and labour intensive due to its perennial nature and long generation period (4–5 years). In addition, tea breeding has been slowed down by the lack of reliable selection criteria. Morphological markers such as leaf shape, dry matter production, biomass partitioning and flesh evenness, or biochemical markers such as total catechin/polyphenol content and caffeine, are used to identify superior tea plants. However, tea breeders are often unable to use markers because they are influenced by environmental factors. To overcome these problems, a limited number of isozyme markers have been used, resulting in less variability (Mondal *et al.*, 2004). With the progress of molecular biology, however, efforts have shifted to using various DNA markers. Molecular markers could be used for marker-aided selection (MAS) and construction of higher-density genetic linkage maps to locate the quantitative trait loci (QTL) of agronomically important traits, quality, and biotic and abiotic resistance characters. Understanding genetic diversity at the molecular level of tea germplasm will mainly help to identify individual tea cultivars through the use of molecular fingerprinting, classify tea genotypes taxonomically using molecular markers, and enhance tea varieties for agronomically important characteristics through MAS.

Biotechnological tools appear to be the ideal choice to avoid problems of conventional tea breeding. Transgenic technology has immense potential for genetic improvement of tea. However, tea plants have been shown low competence for *Agrobacterium*-mediated transformation, as well as low regeneration capacity, because of the occurrence of high levels of polyphenols with germicidal property (Mondal *et al.*, 2004). Genetic transformation of tea through *Agrobacterium tumefaciens* using different explants such as *in vitro*-grown leaves, somatic embryos and embryogenic tissues has been tried (Mondal *et al.*, 2001; Sandal *et al.*, 2007). Somatic embryogenesis is considered one of the most convenient systems to regenerate transformed tea. However, hardening of *in vitro*-grown microshoots has been proven difficult (Mondal *et al.*, 2001). To circumvent this problem, microshoots have been grafted on to the seedling-derived root-stocks of the same cultivar (Mondal *et al.*, 2001). In this technique, a scion from a quality cultivar is grafted onto the root-stock from a drought-tolerant or high-yielding cultivar. Upon grafting, the scion and stock influence each other and thus, the composite plant combines both yield and quality characters, resulting in a significant increase in yield of better quality than either of the non-grafted cultivars (Prakash *et al.*, 1999). Biolistic-mediated genetic transformation (*gene gun* method) is another method used for producing transgenic tea plants. Recently, tea plants expressing stress tolerance genes were developed through this method (Bhattacharya *et al.*, 2006).

Tea plants show a long life cycle, self-incompatibility and high inbreeding depression, so the study of its genetic improvement turns out to be very difficult. Therefore, the isolation and cloning of key functional genes of tea plants, while simultaneously searching for appropriate methods for tea genetic transformation and tissue culture, will become a central part of tea molecular biology in the future. For instance, isolation and cloning of important functional genes of tea plants have a critical significance on elucidating the molecular mechanism of high quality, yield and resistance, as well as genetic manipulation via biotechnological approaches for tea plants. Approximately 25 full-length genes, cloned from tea plants, have been deposited in the GenBank so far. Most of them are important enzymes related to the secondary metabolism, quality and stress tolerance of the tea plant.

IX. ROLE OF PROCESSING, BREWING AND STORAGE ON COMPOSITION OF TEA AND TEA PRODUCTS

A. Tea Manufacture

The manufacturing processes to which the fresh tea leaf is subjected are largely responsible for different types of tea. The major steps involved in the manufacture of different teas are given next. The chemistry of tea manufacture will be discussed later.

Green tea manufacture is characterized by the inactivation of the enzymes, particularly polyphenol oxidase, in the fresh leaves directly after harvest to prevent polyphenol oxidation. For production of Chinese green tea (Kamairi-cha), pan-firing or parching (exposure to dry heat) instead of steaming is employed (Takeo, 1992). With pan-firing the leaves are exposed to much higher temperatures than when steaming is used. Alternatives to steaming and parching have been investigated; microwave-inactivation of enzymes (Gulati *et al.*, 2003; Sharma *et al.*, 2005) and far-infrared irradiation replacing parching (Kim *et al.*, 2006) resulted in increased total catechin content of the leaves. Different types of Japanese green tea, i.e., Ten-cha, Gyokuro, Matcha, and Sen-cha, are obtained by harvesting leaves of tea grown under different degrees of sun exposure, and by varying the degree of firing after drying (Takeo, 1992; Chu, 1997).

For production of oolong tea the leaves are subjected to a moderate level of enzymatic oxidation during processing and drying, resulting in a degree of fermentation ranging between 20 and 80% (Dou *et al.*, 2007). The withered leaves are gently rolled to induce mechanical injury, activating enzymatic reactions. Pan-firing is employed to inactivate the enzymes and terminate fermentation (Takeo, 1992; Dou *et al.*, 2007). Complex oxidation products of the catechins such as theaflavins and thearubigins have been found in oolong tea (Dou *et al.*, 2007), although in this study an accurate determination of the concentration of these compounds was not carried out.

Black tea is fully-fermented and characterised by the presence of theaflavins and thearubigins, responsible for briskness, color, body, and taste of the infusion (Robertson, 1992). Processing entails withering of the leaves after harvest, size reduction, and leaf cell disruption through various means,

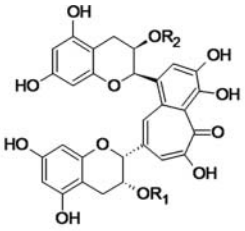
fermentation, and firing (Hampton, 1992). Disruption of the cellular compartmentation brings vacuolar phenolic compounds in contact with cell wall and plastid polyphenol oxidases, initiating oxidation of catechins to theaflavins and thearubigins.

A tea worth mentioning is white tea, an unfermented and minimally processed tea with the delicate white hairs on the leaf intact, giving it a white appearance (Hilal and Engelhardt, 2007). Production of white tea is limited at 600–800 tons annually, making it one of the rarest and most expensive teas (Cooper, 2006). It is a product originally defined by its historic origin, the Chinese province Fujian, but with other countries also producing white tea a definition based on the process or plucking standard, as opposed to origin, is favored (Cooper, 2006; Hilal and Engelhardt, 2007). White tea is made from the new growth buds and young leaves of the plant (*Camellia sinensis* var. khenghe bai hao and *Camellia sinensis* var. fudin bai hao in China), plucked in early spring before the buds are fully opened (Hilal and Engelhardt, 2007). The leaves are sometimes shielded from sunlight during growth to reduce the formation of chlorophyll. The fresh leaves is steamed intact and dried to prevent oxidation (thus white teas lack catechin oxidation products such as theaflavins and thearubigins) (Venditti *et al.*, 2010). White tea has a very light, yellowish infusion compared to the greenish color of green tea (Santana-Rios *et al.*, 2001).

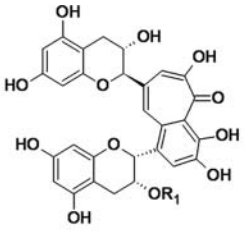
Pu-erh tea, produced and consumed mainly in China, is a microbial fermented tea produced from the leaves of the large-leaf variety (Lu and Hwang, 2008; Wang *et al.*, 2010). The green leaf is parched and partially dried before post-fermentation to make raw pu-erh tea, which is ripened (accelerated ageing in humid conditions) or aged for a very long period. For ageing the raw pu-erh tea is pressed in a cake and stored under natural conditions (Zhang *et al.*, 2011). *Aspergillus niger* is the predominant fungus during post-fermentation (Jeng *et al.*, 2007; Mo *et al.*, 2008; Qin *et al.*, 2012). The long post-fermentation process leads to changes in phenolic composition (Liang *et al.*, 2005; Zhou *et al.*, 2005; Qin *et al.*, 2012). Japanese post-fermented tea is produced either by anaerobic fermentation of green tea or a combination of aerobic and anaerobic fermentation processes. Anaerobic fermentation leads to formation of catechin degradation products, identical to catechin metabolites formed by mammalian intestinal bacteria (Tanaka *et al.*, 2012). Post-fermented tea should not be confused with Kombucha, which is a sweet-sour tea beverage made from tea extract supplemented with sugar and fermented with yeast and acetic acid bacteria (Mo *et al.*, 2008).

B. Chemistry of Tea Fermentation

Changes in the phenolic composition of the leaf introduced by fermentation and the role of catechins as precursors of a complex mixture of oxidation products have been previously reviewed (Robertson, 1992; Haslam, 2003; Drynan *et al.*, 2010; Tanaka *et al.*, 2010). Recent studies have provided advances in understanding the chemistry of black tea thearubigins, which account for 60–70% of the tea solids of the infusion (Kuhnert, 2010; Kuhnert *et al.*, 2010a, 2010b; Drynan *et al.*, 2012). For details of reaction mechanisms and citations of the original research the reader is referred to the relevant reviews, in particular that of Drynan *et al.* (2010) and Tanaka *et al.* (2010).



Theaflavins



Isotheaflavins

		R_1	R_2
Theaflavins	Theaflavin	H	H
	Theaflavin-3-*O*-gallate	Galloyl	H
	Theaflavin-3′-*O*-gallate	H	Galloyl
	Theaflavin-3,3′-*O*-digallate	Galloyl	Galloyl
Isotheaflavins	Isotheaflavin	H	H
	Isotheaflavin-3′-*O*-gallate	Galloyl	H

FIG. 3. Structures of theaflavins and isotheaflavins.

The major dimeric oxidation products in black tea are the theaflavins, theaflavin, theaflavin-3-monogallate, theaflavin-3′-monogallate, and theaflavin-3,3′-digallate (Figure 3), formed through a condensation reaction between (-)-EGC and (-)-EC and their gallates. Isotheaflavins, formed with (+)-gallocatechin or (+)-catechin as one of the units, are detected in low quantities (<0.01% DW) in black tea. It is believed that the 2,3-*trans* stereochemistry of these precursors as opposed to the 2,3-*cis* stereochemistry of (-)-EC and derivates makes them less susceptible to the condensation reaction, explaining their low quantities compared to theaflavins. These condensation products possess a benzotropolone chromophore, responsible for their yellow-orange color (Drynan *et al.*, 2010). Theaflavins correlate with the brightness of the infusion (Ngure *et al.*, 2009) and are used as a quality parameter of black tea (Owuor *et al.*, 1986; Wright *et al.*, 2002; Muthumani and Kumar, 2007). In spite of giving a mouth-coating, astringent, and long-lasting oral sensation at the very back of the throat at much lower thresholds than their precursors, the contribution of theaflavins to the astringency of black tea infusions was estimated at less than 0.1% (Scharbert *et al.*, 2004b).

Using model fermentation conditions Tanaka *et al.* (2010) demonstrated that oxidation of theaflavin can lead to theanaphtoquinone as major product and other reddish-brown products, i.e., bistheaflavins and dehydrotheaflavin. Minor condensation products of the catechins containing the benzotropolone moiety are theagallins (red), theaflavates (yellow to orange-red) and theaflavic acids (red). Results of model fermentations showed that theaflavic acids, condensation products of catechin quinones with gallic acid, are ultimately converted to thearubigins, explaining why they are present in trace quantities in black tea. Evidence of tri- and dibenzophenones, i.e., theaflavin type trimers formed through coupling of theaflavins via galloyl

ester groups with catechins, have been provided by model studies. Two compounds, theadibenzotropolone A and theadibenzotropolone B were quantified at 220 and 70 μg/kg DW, respectively, in black tea leaf (Drynan *et al.*, 2010; Tanaka *et al.*, 2010).

Compounds without the benzotropolone moiety but with an intense yellow are the theacitrins, containing a three-fused ring system (Drynan *et al.*, 2010). The formation of many other oxidation products of catechins, including unstable intermediates such as dehydrotheasinensins, has been demonstrated, using model fermentation systems. Dehydrotheasinensins, which are unstable quinone dimers of EGC and EGCG, are converted to theasinsensins (previously known as bisflavanols) during exposure to high temperatures (80°C) and to oolongtheanin (Tanaka *et al.*, 2010). They are also present in fresh green tea leaf, but are mainly found in oolong tea (Drynan *et al.*, 2010; Sang *et al.*, 2011). These compounds do not contain the benzotropolone moiety and are colorless (Tanaka *et al.*, 2003).

Thearubigins, the major compounds responsible for the color and strength of black tea infusions, are a poorly-defined heterogeneous mixture of catechin oxidation products (Robertson, 1992). Based on MALDI-TOF-MS analysis Drynan *et al.* (2012) demonstrated that the caffeine-precipitated SII thearubigin fraction is composed of a large number of polyhydroxylated oligomers of catechins and catechin gallates in redox equilibrium with their quinone counterparts. These oligomers are molecular weight compounds of less than 2100 $gmol^{-1}$, contrary to the previously suggested polymeric nature of the heterogeneous fraction.

The extent to which conversion of the catechins and their oxidation products takes place during tea production will depend on processing conditions. It is the most extensive in black tea production with cut-tear-curl (CTC) processing being more destructive than orthodox processing (Crozier *et al.*, 2009). Analysis of a large number of commercial green and fermented teas enabled Lin *et al.* (2008) to show that the partially fermented teas contained approximately half of the EGCG content of the green teas, while the black teas had only traces of EGCG, but contained theaflavins. Highly overfermented black tea contained only trace quantities of theaflavins, and flavonol glycosides.

Ripened pu-erh tea was shown to have substantially lower quantities of EGCG, EGC, ECG, and quinic acid than aged pu-erh tea, while the opposite was found for gallic acid (Ku *et al.*, 2010). Degradation of the gallate ester bond is considered the main metabolic mechanism during the initial stage of post-fermentation (Qin *et al.*, 2012). New compounds formed in the post-fermentation process of pu-erh tea were 8-*C* substituted flavan-3-ols, puerins A and B, and two cinchonain-type phenols (Zhou *et al.*, 2005). Based on spectrophotometric methods, low quantities of theaflavins and thearubigins are present in pu-erh tea (Xie *et al.*, 2009).

C. Composition of Manufactured Tea

Peterson *et al.* (2005) collated and evaluated data in literature, based on water extraction for compilation of a database of the content of the major flavonoids in different types of tea. Quantitative data for the composition of different commercial teas are normally characterised by large variation within and between types (Tables 3 and 4), as numerous factors, ranging from variety to processing as described in the previous sections, play a role. Comparison of data between different studies is rarely valid as sample preparation conditions differ, skewing data. Sample preparation varies, amongst others, in the solvents used, time and temperature of extraction, particle size and solid:solvent ratio, all factors that could affect the extraction efficacy of tea constituents. Friedman *et al.* (2006), employing a large number of samples of different types of tea including green and black teas, demonstrated that extraction of catechins and theaflavins are more effective with 80% aqueous ethanol at 60°C for 15 min followed by sonication for 5 min and re-extraction of the residue twice more with the same solvent, than extraction with freshly boiled water (ca 90°C) and stirring for 5 min (Table 5). Almost no theaflavins were found in green tea, showing that heating of the fresh leaf to denature enzymes is effective in preventing enzymatic oxidation. On the other hand

TABLE 3
Concentration range of individual catechins in major types of processed tea

Tea	Extraction procedure	C	EC	ECG	EGC	EGCG
Green tea (China, Taiwan, Japan; $n = 14$)[a]	1% (w v^{-1}), 75% ethanol at 60°C, 30 min	nd – 2.4	1.3 – 7.3	4.6 – 14.0	9.9 – 94.7	14.9 – 48.2
Green tea (China; $n = 160$)[b]	ISO standard 14502-1	0.9	3.8	14.2	5.6	52.2
Oolong tea (China, Taiwan; $n = 13$)[a]	1% (w v^{-1}), 75% ethanol at 60°C, 30 min	0.6 – 3.0	0.8 – 6.2	0.6 – 9.2	7.8 – 115.0	2.9 – 30.7
Green tea ($n = 24$)[c]	0.6% (w v^{-1}), boiling water, 5 min	nd – 33.2	0.1 – 2.6	1.2 – 27.1	nd – 14.2	2.2 – 53.6
Black tea ($n = 32$)[c]	0.6% (w v^{-1}), boiling water, 5 min	nd – 8.1	0.4 – 6.3	1.7 – 26.8	nd – 10.3	0.6 – 28.4

Values are given as mg g^{-1} DW.
Adapted from [a]Lin *et al.* (2003), [b]Xu *et al.* (2012) and [c]Friedman *et al.* (2005); nd, not detected.

TABLE 4
Concentration range of individual and total theaflavins in black tea

Compound	mg g^{-1} DW
Theaflavin	nd – 5.4
Theaflavin-3-O-gallate	nd – 2.1
Theaflavin-3′-O-gallate	nd – 0.9
Theaflavin-3, 3′-O-digallate	nd – 6.4
Total theaflavins	0 – 8.8

[a]Number of tea analyzed = 32.
Adapted from Friedman *et al.* (2005); nd, not detected.

studies comparing solvents showed 15% ethanol to be optimal for extraction of catechins (Wang *et al.*, 2000b) or boiling water more effective than 80% methanol or 70% ethanol (Khokhar and Magnusdottir, 2002). In a comparison of simulated preparation conditions of tea drinking (1 g tea infused in 100 ml water at >95°C for 5 min; solid:solvent ratio of 0.1:10) with simulated industrial extraction conditions, i.e., 30 min sonication-assisted extraction of ground tea leaves with 80% aqueous methanol in a solid:solvent ratio of 1:10 (Unachukwu *et al.*, 2010), hot water extraction of different green teas was, in most cases, more effective in extracting catechins than aqueous methanol (Table 6). For the different white teas analysed the opposite was observed. Values obtained with water extraction varied between 21.38 to 228.20 mg g^{-1} DW for the different samples of green tea and 14.40 to 369.60 mg g^{-1} DW for white tea, constituting on average 9.97% and 8.20% of the respective teas. When using aqueous methanol the average total catechin content of green and white teas constituted 6.77% and 7.62%, respectively.

Zhao *et al.* (2011) employed finely powdered samples of commercial green pu-erh, green and white teas for extraction with 60% aqueous methanol while sonicating for 60 min at room temperature. Of the individual catechins determined, EGCG was present at the highest level in white tea (169.77 ± 4.76 mg g^{-1} DW), green tea (144.21 ± 29.12 mg g^{-1} DW) and green pu-erh tea (88.33 ± 37.54 mg g^{-1} DW), followed by EGC in green and white teas and EC in green pu-erh tea.

The large variation in catechin content of a specific type of tea underscores the need for caution in making blanket claims regarding high content values without the support of quantitative data for specific batches of tea.

D. Increasing Phenolic Content by Controlling Brewing Conditions

Brewing conditions employed by the consumer are largely determined by taste preference. Manipulation of brewing conditions to enhance the polyphenol content of the infusion or brew has thus limited value considering excessive astringency and bitterness at high concentrations of catechins and their oxidation products. Usually tea would be prepared by steeping of 2 g loose tea or one tea bag per cup in freshly boiled water for 2–5 min. Black tea at a standard 1% infusion typically contains 42 mg total catechins, whereas green tea infusion contains 136 mg total catechins (recalculated from compiled data by Peterson *et al.*, 2005).

Investigation of the extraction of tea constituents started prior to the interest in these compounds for their health-promoting properties, and was largely governed by their role in sensory quality. Using first-order rate law plots of kinetic data diffusion of leaf constituents in the leaf matrix was shown to be the rate-limiting step (Spiro and Siddique, 1981; Spiro and Jago, 1982; Price and Spitzer, 1993, 1994). As a result factors that increase their rate constant therefore increase the rate of extraction and thus extraction efficiency. Temperature plays a critical role in the dissolution of and extent to which the water-soluble components, including the catechins and their oxidation products, are released from the leaf (Spiro and Siddique, 1981; Price and Spitzer, 1993, 1994). Extraction efficiency of catechins (Khokhar and Magnusdottir, 2002) from black tea during a 5-min infusion period under standard preparation conditions increased substantially when the temperature was increased from 60°C to 100°C. At 60°C the extraction of EGCG, EGC and C was ca 50, 70, and 85%, respectively, of that obtained at 100°C. A study on green tea, investigating infusion time (3–7 min) and temperature (70–100°C) showed that the highest concentrations of flavanols in the infusion tea are achieved after 7 min steeping at 100°C (Zimmermann and Gleichenhagen, 2011). A study of the infusion rate of the four major tea flavanols from Japanese green tea indicated that the ungallated compounds, EC and EGC, infused faster than the gallated catechins, ECG and EGCG (Price and Spitzer, 1994). Thearubigins extracted faster than theaflavins (Spiro and Siddique, 1981).

Since diffusion within the leaf matrix is rate-limiting, leaf particle size affects the strength of an infusion, given a finite extraction time (Price and Spiro, 1985). The rate constant of theaflavins increased by a factor of 2.2 as leaf size decreased from 850–1000 μm to 500–600 μm. In addition, factors such as the pH of the water and the presence of salts were found to affect extraction of tea consitutents (Spiro *et al.*, 1987; Couzinet-Mossion *et al.*, 2010; Zimmermann and Gleichenhagen, 2011). Alkaline conditions increased the rate constant of theaflavins, which is accounted for by the partial dissociation of theaflavins at higher pH and the greater diffusion coefficient of the resulting ionic salt (Spiro *et al.*, 1987). Alkaline conditions are, however, conducive to degradation of the catechins (Zhu *et al.*, 1997; Chen *et al.*, 2001). Increasing the calcium content of acidic media lowers the extraction of polyphenols and caffeine (Couzinet-Mossion *et al.*, 2010). Addition of citric acid to water slightly improved extraction of catechins from white tea, but not from green tea (Rusak *et al.*, 2008). Other factors that influence the composition of a cup of tea is the type of tea, use of loose tea versus tea bags (Astill *et al.*, 2001; Rusak *et al.*, 2008), and even tea bag material (Yao *et al.*, 2006). Indian orthodox teas have lower rate constants for theaflavins than Kenian CTC teas (Price and Spiro, 1985). Agitation of tea bags (Astill *et al.*,

TABLE 5
Content of 5 individual catechins [catechin (C), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG)], total catechins (TC); 4 individual theaflavins [theaflavin (TF), theaflavin-3-gallate (TF3G), theaflavin-3′-gallate (TF3′G), theaflavin-3,3′-gallate (TF3,3′G)], and total theaflavins (TFS) in black and green tea extract prepared with 80% ethanol/water or water

		C	EC	ECG	EGC	EGCG	TC	TF	TF3G	TF3′G	TF3,3′G	TFS
Black tea	Water extract[a]	nd-8.1	0.3–6.3	1.7–26.8	nd-10.3	0.5–28.4	5.4–69.5	nd-5.4	nd-2.1	nd-0.9	nd-6.4	0–8.1
	80% ethanol/water[b]	nd-4.7	0.6–7	3.8–67.1	nd-6.1	0.8–47.8	9.6–111	0.4–9.1	nd-5.9	nd-3.8	0.6–10.9	3.9–20.7
Green tea	Water[a]	nd-7.9	0.1–2.6	1.2–27.1	nd-13.9	2.2–53.6	4.4–100	nd-0.2	nd	nd	nd	0.2
	80% ethanol/water[b]	nd-4.8	tr–3.2	2.4–40.7	nd-13.9	7–74	12.3–136.3	nd	nd	nd	nd	0

Values are given in mg g^{-1} DW ($n = 3$).

Adapted from Friedman *et al.* (2006); tr = trace; nd = not detected.

[a]Extraction conditions - solid:solvent ratio of 0.06:10 (w v^{-1}) and boling water for 5 min with stirring; [b]Extraction conditions - solid:solvent ratio of 0.3:10 (w v-1), 15 min at 60°C and 5 min sonication at room temperature.

TABLE 6
Range and mean of total catechin contents (TC) in green and white tea

Solvent extract	TCC	Mean
Green tea		
Water[a]	21.38–228.2	109.71
80% Methanol/water[b]	32.23–141.24	67.23
White tea		
Water[a]	14.4–369.6	82.01
80% Methanol/water[b]	47.16–163.94	76.15

Values are given as mg g^{-1} DW. Tea sample sizes: $n = 19$ (green tea) and 8 (white tea).

[a] Extraction conditions - solid:solvent ratio of 0.1:10 (w v^{-1}) and 95 – 100°C for 5 min; [b]Extraction conditions - solid:solvent ratio of 1:10 (w v^{-1}) and 30 min sonication at room temperature.

Adapted from Unachukwu *et al.* (2010).

2001), microwave-assisted extraction (Spigno and De Faveri, 2009), and combining steeping of the tea bag in freshly boiled water, followed by heating in a microwave (Vuong *et al.*, 2012) improve extraction efficiency.

E. Production of Extracts

Instant tea, made from black tea, has been on the U.S. market for many years as a convenient alternative to loose tea and tea bags. In this case yield and clarity of the instant tea in cold water when reconstituted as a beverage have been the subject of many patents (Pintauro, 1977). Greater awareness by consumers of the health benefits of tea catechins has stimulated the development of green tea extracts, recently estimated at a market value of 44 million USD and predicted to grow by more than 13% over the next 7 years (Byrne, 2010). Green tea extracts and derived products, in particular catechin-enriched extracts and highly purified extracts containing mostly EGCG, find a myriad of uses ranging from food ingredients to cosmetic use. However, products do not always deliver the quantities of catechins as promised. Analysis of a number of dietary supplements, containing only green tea extract, showed that the EGCG content of the products varied between 3.8 and 44.4% (Seeram *et al.*, 2006). For the same two products (diffent manufacturers) their corresponding ECG contents were 1.1 and 11%. Of the ten products analyzed five provided information about EGCG on the label, but only two contained the claimed amount. In one instance the label claimed 55% while the actual content was 27.1%.

Apart from the selection of the raw material extract manufacturing processes are critical in ensuring product consistency. Factors important for the efficient extraction of tea constituents when preparing a cup of tea remain relevant in industrial processing, albeit driven by technological considerations and economy of scale. The basic steps in the production of instant teas and powdered extracts are extraction of the tea leaves followed by concentration and drying. Yield, product solubility, clarity when reconstituted in a beverage and degree of enrichment are all factors that govern processing conditions. Details of processes including those used for enrichment and purification of catechins can be found in a review by Vuong *et al.* (2010). The following section provides a brief summary of a few studies, illustrating how extraction conditions could be used to manipulate yield and composition of the extract.

Extraction efficiency of more than 90% of green tea catechins, comprising ca 19% of the dry tea leaf can be achieved at 80°C after 20–30 min extraction, using a 1:40 tea:water ratio. Prolonging the extraction time led to a decrease in content, which was attributed to degradation (Perva-Uzunalić *et al.*, 2006). Another study recommended the use of 50% ethanol when extracting dry tea leaf, 75% ethanol when extracting fresh leaves or a water temperature of 80°C or less (Liang *et al.*, 2007). Vuong *et al.* (2011) achieved optimum extraction of catechins with water at pH 6 when tea particles of 1 mm size were extracted in a 1:50 tea:water ratio at 80°C for 30 min. A lower tea:water ratio and smaller particle size offered no advantage, while a higher pH and temperature resulted in epimerization and degradation. Considering the most efficient use of water and the lowest drying cost, a two-step extraction procedure, i.e., once at a tea:water ratio of 1:12 and once at a tea:water ratio of 1:8, was found to give the best extraction.

Extraction temperature not only plays a critical role in the extraction efficiency and stability of the catechins, but it can also be used to enrich the extract in certain compounds. Extraction with water at 5°C results in the enrichment of EGC and EC (77% of total catechins extracted), while at 80°C extraction of EGCG increased substantially, accounting for 43% of the total catechins (Vuong *et al.*, 2011). Labbé and co-workers earlier proposed a two-step extraction procedure to fractionate EGC and EGCG, based on their different solubilization kinetics as affected by temperature and extraction time (Labbé *et al.* 2006, 2008; Bazinet *et al.*, 2007). Extraction at 50°C for 10 min would allow enrichment in EGC (78.9% of total catechins) and subsequent extraction of the same leaves at 80°C for 10 min (or 90°C for 10 min) would lead to enrichment in EGCG (47.6% of total catechins). The benefit of this procedure is that no organic solvent, precipitation or membrane separation process is needed. By applying electrodialysis using a UF-1000 Da membrane further enrichment could be achieved (Labbé *et al.*, 2005).

Hu *et al.* (2009) also made use of the different solubilisation kinetics of the catechins to prepare green tea extract enriched in EGC or EGCG, but in their case solvent composition in addition to temperature and time of extraction were manipulated. Extraction of green tea with 75% ethanol at 30°C for 10 min (1:60 tea:solvent ratio) and subsequent extraction of the "spent" leaves with 35% ethanol at 90°C for 10 min at the same solid: solvent ratio gave EGC and EGCG-enriched extracts, respectively.

F. Stability During Storage of Processed Tea Leaf, Infusions, and Tea Products

Black tea is prone to deterioration and loss of value during storage (Stagg, 1974; Cloughley, 1981) as a result of post-manufacture reactions especially when its moisture content is above 4 ± 1% (Hampton, 1992). Residual polyphenol oxidase activity is responsible for loss of theaflavins and an increase in thearubigins (Sanderson, 1972). Friedman *et al.* (2009b) demonstrated a progressive decrease in the total catechin levels of commercial green tea bag products, stored for 6 months in the dark at 20°C in their original retail packaging. Their average loss in total catechins during this period was 32%. EGCG and ECG decreased by 28 and 51%, respectively, while EC remained relatively stable, decreasing by 8.6%. None of the teas investigated contained EGC and C.

The stability of green tea powdered extract is determined by storage conditions with degradation accelerated by an increase in temperature and humidity (Li *et al.*, 2011). When added to ready-to-drink beverages factors such as product formulation, pH, severity of heat processing, storage conditions and even the composition of the extract affect stability of the catechins (Wang and Helliwell, 2000; Chen *et al.*, 2001; Su *et al.*, 2003; Sang *et al.*, 2005; Labbé *et al.*, 2008). Acidification of the beverage increases their stability, while the presence of ascorbic acid and citric acid enhanced their degradation (Su *et al.*, 2003). However, ascorbic acid increased the stability of the catechins at pH 7.42 (Chen *et al.*, 1998). High temperatures required for microbial stability lead to epimerization, but it is less severe when heating took place under ultra high temperature (UHT) conditions due to shorter heat exposure. Epimerization and degradation of tea catechins follow first-order reactions and the reaction rate constants followed the Arrhenius equation (Wang *et al.*, 2006). The large amounts of GCG found in ready-to-drink teas were attributed to epimerization of EGCG (Chen *et al.*, 2001). The catechins were more stable than theaflavins under the same conditions (Su *et al.*, 2003). Both were shown to have poor long-term stability with approximately 50% degraded within the first month of storage at room temperature. Use of extracts enriched in catechins and low temperatures seems to enhance product stability during storage (Sang *et al.*, 2005; Labbé *et al.*, 2008; Bazinet *et al.*, 2010). Kim *et al.* (2011) demonstrated that glass bottles are superior to polyethylene terephthalate (PET) and retortable pouches for retaining EGCG, EGC and EG in green tea beverage stored at 3°C in the absence of light for 12 weeks.

Limited information is available on the stability of green tea extract when used as functional ingredient in other food products. A few studies investigated the stability of the catechins during bread and biscuit making, demonstrating that epimerization and degradation occur simultaneously (Ananingsih *et al.*, 2011; Sharma and Zhou, 2011). Substantial losses in EGCG and ECG were observed (Sharma and Zhou, 2011). Degradation and epimerization of EGCG during bread baking followed first-order kinetics, with the activation energy unchanged from that in an aqueous system (Wang *et al.*, 2008). In addition to the high temperature during baking and the alkaline pH of the dough which promote loss of catechins, interaction with other components of the dough may also affect their stability. EGCG and ECG were stable in frozen dough for 9 weeks or 4 days at room temperature and were retained in high levels in freshly baked bread (Wang and Zhou, 2004). As the use of green tea extracts in foods increases more research is needed to provide a greater understanding of their stability during processing and storage so that consumers could be confident that the functional ingredient is present.

CONCLUSIONS

Tea catechins, the most abundant phenolic compounds in the tea plant, are of interest due to their role in the sensory and health properties of tea. This has led to the development of green tea extracts for use as functional ingredients by the food industry. High levels of these compounds are thus desired, challenging scientists at both the pre- and post-harvest stages of tea production and processing. It has been reviewed here that the composition of the tea leaf, and thus tea quality, is influenced by many pre-harvest factors such as the genetic background of the plant, geographical origin, horticultural and harvesting practices, and environmental conditions. Once harvested, processing, brewing and storage conditions influence the phenolic composition and quality of tea infusions as well. Optimizing both pre- and post-harvest techniques together with transgenic approaches will undoubtedly provide us in the future with the key to develop new varieties with improved polyphenol and health properties to be used in tea manufacturing and preparation of tea infusions and other tea products.

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