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Green tea catechins during food processing and storage: A review on stability and detection

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Green tea catechins can undergo degradation, oxidation, epimerization and polymerization during food processing. Many factors could contribute to the chemical changes of green tea catechins, such as temperature, pH of the system, oxygen availability, the presence of metal ions as well as the ingredients added. Several detection methods have been developed for tea catechin analysis, which are largely based on liquid chromatography (LC) and capillary electrophoresis (CE) methods for getting a good separation, identification and quantification of the catechins. Stability of green tea catechins is also influenced by storage conditions such as temperature and relative humidity. The stability of each catechin varies in different food systems and products. Pseudo first-order kinetic model has been developed and validated for the epimerization and degradation of tea catechins in several food systems, whereas the rate constant of reaction kinetics followed Arrhenius equation.

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1. Introduction

Stability of green tea catechins, the phytochemical product produced from tea leaf (*Camellia sinensis*), has been under study for several decades to determine their chemical changes during food processing. Tea catechins contained in green tea are higher than black tea and oolong tea because there is no fermentation process occurring during the manufacture of green tea (Toschi et al., 2000). During fermentation of black tea, polyphenol oxidase in the tea leaves catalyzes the oxidation of the majority of catechins into theaflavin, hence reduces its catechins content (Friedman, Levin, Choi, Lee, & Kozukue, 2009).

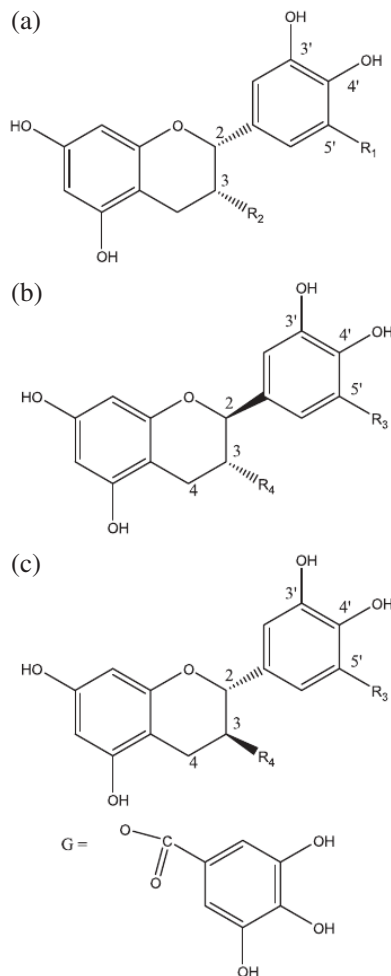
Scientific evidences to support the health benefits of green tea consumption begin to appear. Those benefits include improving blood flow, preventing cardiovascular disease, eliminating various toxins and improving resistance to various diseases (Afaq, Adhami, Ahmad, & Mukhtar, 2004). These might be due to green tea containing catechins which have anti-oxidative, anti-carcinogenic, anti-microbial, anti-viral, anti-inflammatory and anti-diabetic properties (Khan & Mukhtar, 2007; Lakenbrink, Lapczynski, Maiwald, & Engelhardt, 2000; Zaveri, 2006). In addition, green tea catechins have other pharmaceutical activities such as antihypertensive and hypolipidemic (Chan et al., 1999; Henry & Stephens-Larson, 1984). Owing to the health benefits, green tea consumption is increasing, which is reflected by its annual growth rate of ca. 4.5% (FAO, 2008).

The major nutraceutical compounds in green teas are tea catechins, which are flavonols. Flavanols are a class of flavonoids which are polyphenols. Green tea is rich in flavanols (300–400 mg/g) which are of interest to human health (Dubick & Omaye, 2007). Tea catechins have the most effective antioxidant activity compared to other tea polyphenols. The major green tea catechins are (–)-epigallocatechin gallate (EGCG), (–)-epicatechin gallate (ECG), (–)-epigallocatechin (EGC) and (–)-epicatechin (EC). These epicatechins can change to their epimers that are non epicatechins, i.e. (–)-gallocatechin gallate (GCG), (–)-catechin gallate (CG), (–)-gallocatechin (GC) and (±)-catechin (C) (Fig. 1). EGCG is the most abundant and active catechin and it is usually used as a quality indicator (Lakenbrink et al., 2000; Wang & Helliwell, 2000; Wang, Zhou, & Jiang, 2008a). In addition, green tea contains other polyphenols such as gallic acid, quercetin, kaempferol, myricetin and their glycosides, but at lower concentration than EGCG (Dubick & Omaye, 2007; Sakakibara, Honda, Nakagawa, Ashida, & Kanazawa, 2003).

Tea catechins are an efficient free radical scavenger due to their one electron reduction potential. Antioxidant activity as hydrogen or electron donors is determined by this reduction potential of free radicals. A lower reduction potential has a tendency to lose electron or hydrogen (Higdon & Frei, 2003). The rate of reaction with free radicals and the stability of the resulting antioxidant radicals contribute to the reactivity of antioxidant. Guo et al. (1999) reported the scavenging ability of tea catechins on superoxide anions ($O_2^{\cdot-}$), singlet oxygen (1O_2), the free radicals generated from 2,2P-azobis(2-amidinopropane) hydrochloride (AAPH) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. They suggested that the scavenging ability of EGCG and GCG was higher than that of EGC, GC, EC and C due to their gallate group at 3

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Component	Abbrev.	R ₁	R ₂	R ₃	R ₄
(-)-Epigallocatechin gallate	(-)-EGCG	OH	G	-	-
(-)-Epicatechin gallate	(-)-ECG	H	G	-	-
(-)-Epigallocatechin	(-)-EGC	OH	OH	-	-
(-)-Epicatechin	(-)-EC	H	OH	-	-
(-)-Gallocatechin gallate	(-)-GCG	-	-	OH	G
(-)-Gallocatechin	(-)-GC	-	-	OH	OH
(-)-Catechin gallate	(-)-CG	-	-	H	G
(+)-Catechin	(+)-C	-	-	H	OH

Fig. 1. General chemical structures of green tea catechins: (a) epi catechins; (b) non-epi catechins and (c) (+)-catechin.

position of C ring. While abilities to scavenge free radicals for EGC and GC were stronger than EC and C because of a hydroxyl group at the 5' position of B ring. GCG was more stable than EGCG because it has smaller steric hindrances. In addition, the stability of GC and C was better than EGC and EC. A summary of the scavenging activity of tea catechins on different free radicals/ROS is shown in Table 1.

It is important to understand the stability of green tea catechins in foods during processing and storage in order to gain the optimum health benefits from them. The level of tea catechins could be easily reduced as a result of epimerization and degradation during processing (Wang et al., 2008a). The catechin stability in different food systems could be

Table 1
Scavenging activity of tea catechins on free radicals/ROS.

Free radicals/ROS	The order of scavenging activity	References
Singlet oxygen	EC, C EGC, GC EGCG, GCG, EGCG ECG EGC EC C	Guo et al., 1999 Mukai, Nagai, & Ohara, 2005
Hydroxyl radical	ECG EGCG EC GC EGC C ECG EC EGCG EGC	Wiseman, Balentine, & Frei, 1997 Guo, Zha, Li, Shen, & Xin, 1996
Lipid peroxyl radical	ECG=EGCG=EC=C EGC	Salah et al., 1995
ABTS ^{•+} radical cation	ECG EGCG EGC EC=C ECG EGCG EGC EC	Salah et al., 1995 Higdon & Frei, 2003
DPPH [•] radical	EGCG=ECG EGC EC	Nanjo et al., 1996
AAPH	EGCG EGC EC	Guo et al., 1999

influenced by pH, temperature, oxygen availability, the presence of metal ions, and also concentration of other active ingredients (Guo et al., 1999; Kumamoto, Sonda, Nagayama, & Tabata, 2001; Komatsu et al., 1993; Sang, Lee, Hou, Ho, & Yang, 2005).

In this paper, the results of and mechanisms behind the chemical changes of green tea catechins in various food systems are reviewed. A summary of the detection methods of tea catechins will also be presented and discussed. The stability of catechins during processing and storage in a number of real food products will be discussed, together with kinetic modeling results.

2. Stability of catechins: epimerization and degradation

Epimerization is the conversion of tea catechins to their corresponding isomers. The identified epicatechins in green tea i.e. EGCG, EGC, ECG, and EC are in *cis* structure. They can convert to their epimers that are non-epicatechins, i.e. GCG, GC, CG, and C, respectively (Wang et al., 2008a; Chen & Chan, 1996). This epimerization between pair catechins is reversible. The chemical structures of epicatechins and non-epicatechins only differ between 2R, 3R (2, 3-*cis*, epi-form) and 2S, 3R (2, 3-*trans*, nonepiform). Fig. 2 illustrates the reversible epimerization between EGCG and GCG.

Epimerization can occur at high temperature (Wang & Helliwell, 2000). It has been recognized that catechins undergo epimerization at the C-2-position in hot aqueous solution. This epimerization can change the epistructured catechin to non-epi-structured catechin and vice versa. Wang et al. (2008a) reported that the concentration of catechins decreased while their isomers increased as the temperature increased. Degradation of catechins was evident as there was a declining trend in total catechins with increasing temperature. Many researchers have found that tea catechins could convert to their corresponding epimers in traditionally brewed tea infusion and canned tea drinks during brewing, production, and storage (Chen, Zhu, Tsang, & Huang, 2001; Zhu, Zhang, Tsang, Huang, & Chen, 1997). Tea catechins undergo many chemical changes such as oxidation and epimerization during the course of the brewing processes. As a result, epimerization of the catechins is thought to be one of the most important reactions in the manufacture of green tea (Wang & Helliwell, 2000).

2.1. Effect of pH and temperature

The stability of tea catechins is pH and temperature dependent. Tea catechins in aqueous solutions are very stable when pH is below 4, whereas they are unstable in solutions with pH > 6. In addition, storage temperature affects the stability of tea catechins significantly even at ambient conditions (Chen et al., 2001; Komatsu et al., 1993; Su, Leung, Huang, & Chen, 2003; Wang & Helliwell, 2000; Wang, Zhou, & Wen, 2006). Stability studies on catechins in green tea infusion have shown that epimerization could be observed at 40 °C over prolonged storage. Therefore, not only temperature, but also heating time influenced the epimerization of catechins in green tea infusions (Wang & Helliwell,

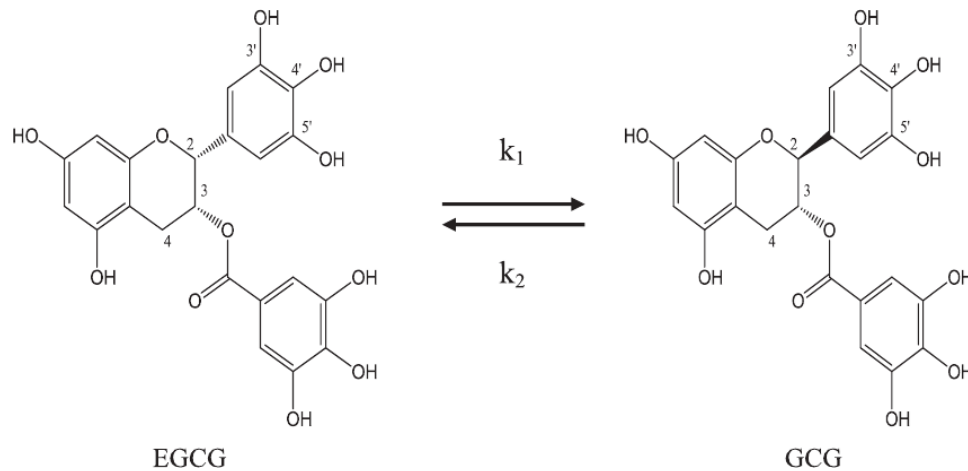


Fig. 2. Epimerization of epistructured catechin EGCG to non-epistructured catechin GCG and vice versa; k_1 rate constant from EGCG to GCG; k_2 , rate constant from GCG to EGCG.

2000). Komatsu et al. (1993) reported that the degradation and epimerization of tea catechins could occur simultaneously in thermal processes. Wang, Zhou and Wen (2006), and Wang, Zhou and Jiang (2008a) demonstrated that both epimerization and degradation followed pseudo first-order kinetics and the dependence of the kinetic constants on temperature could be described by the Arrhenius equation.

2.2. Effects of oxygen concentration and metal ions

Catechin stability is influenced by oxygen concentration, the presence of free radicals as well as metal ions. Sang et al. (2005) reported that higher oxygen levels and low concentration of antioxidants increased catechin oxidation. Under low O_2 concentration (flushed with N_2) at 37 °C and pH 7.4, EGCG remained stable with only 5% degradation after 6 h. Epimerization of EGCG to GCG was suspected as the cause of this degradation because dimers as an oxidation product of EGCG were not shown. In contrast, in the normal condition without N_2 flushing, aqueous solution of EGCG at 37 °C and pH 7.4 was drastically degraded for 90% after 2 h and furthermore, no EGCG was left after 6 h. They also reported that the presence of ethylenediaminetetraacetic acid (EDTA) enhanced catechin stability, which was suggested to be due to that the metal ions catalyzed the auto-oxidation of EGCG.

Metal ions would affect antioxidative activity of catechins by their binding to the catechins. Catechins react with metal ions to form metal complexes. Kumamoto et al. (2001) reported that antioxidative activity of EGCG increased by the presence of Cu^{2+} and Mn^{2+} , however Fe^{2+} reduced that activity. Metal ions bound to EGCG and changed its oxidation potential. Formation of phenoxy radical easily occurs at ECG and EGCG due to their gallate group. The presence of Cu^{2+} and Mn^{2+} assists the reaction and increases the antioxidative activities of catechins, hence the use of catechins with Cu^{2+} is favorable as an antioxidant. Kumamoto et al. (2001) showed that metal ions preferentially bound to the gallate group of ECG and EGCG. Ryan and Hynes (2007) measured electron transfer of Fe^{3+} to EGCG and ECG using UV-visible spectrophotometry. They showed the powerful antioxidant properties of the ligands with that one molecule of either EGCG or ECG reduced four Fe^{3+} species.

3. Detection methods of tea catechins

In recent years, many methods of analysis have been developed for determining catechins contents in green tea and food products

containing tea catechins. Liquid chromatography (LC) and capillary electrophoresis (CE) are the most cited techniques for catechin separation, identification and quantification (Dalluge & Nelson, 2000). LC systems are typically coupled with UV-Vis (Carando, Teissedre, & Cabanis, 1998; Li, Fong, Singletary, & Fitzloff, 2002; Wang, Helliwell, & You, 2000; Zuo, Chen, & Deng, 2002), diode array (DAD) (Bronner & Beecher, 1998; Goto, Yoshida, Kiso, & Nagashima, 1996), electrochemical (ED) (Kolouchova-Hanzlikova, Melzoch, Filip, & Smidrkal, 2004; Kumamoto, Sonda, Takedomin, & Tabata, 2000; Lee, Prabhu, Meng, Li, & Yang, 2000; Yang, Arai, & Kusu, 2000), mass spectrometry (MS) (Chang & Wu, 2011; Dalluge, Nelson, Thomas, Welch, & Sander, 1997; Del Rio et al., 2004; Flamini, 2003; Poon, 1998), chemiluminescence (Nakagawa et al., 1997; Nakagawa & Miyazawa, 1997), fluorescence (FD) (Carando et al., 1998; Gürbüz et al., 2007; Rodriguez-Delgado, Malovaná, Pérez, Borges, & Montelongo, 2001; Tsanova-Savova, Ribarova, & Gerova, 2005; Vinas, Lopez-Erroz, Marin-Hernandez, & Hernandez-Cordoba, 2000), and Photo Diode Array (PDA) (Sharma & Zhou, 2011; Wang & Zhou, 2004; Zuo et al., 2002) detectors. Two modes of capillary electrophoresis, i.e. capillary zone electrophoresis (CZE) (Horie, Mukai, & Kohata, 1997) and micellar electrokinetic capillary chromatography (MEKC) (Aucamp, Hara, & Apostolides, 2000; Bonoli, Pelillo, Toschi, & Lercker, 2003; Horie & Kohata, 2000; Larger, Jones, & Dacombe, 1998; Nelson, Thomas, Wise, & Dalluge, 1998; Stach & Schmitz, 2001; Weiss & Anderton, 2003; Worth, Wießler, & Schmitz, 2000), have been employed for the determination of catechins. Both modes of capillary electrophoresis are based on ultraviolet detection. Additional analytical techniques, such as gas chromatography (Soleas, Yan, & Goldberg, 2001), thin-layer chromatography (TLC) (Glavnik, Simonovska, & Vovk, 2009; Jerez, Touriño, Sineiro, Lluís, & Núñez, 2007; Li, Tanner, & Larkin, 1996), paper chromatography, spectrophotometry (He et al., 2009), biosensing, chemiluminescence, nuclear magnetic resonance (NMR) spectroscopy and Fourier Transform-Near Infrared Spectroscopy (FT-NIR) have also been utilized for the determination of catechins. A summary of the various catechin detection methods and the food systems for which they have been used is shown in Table 2.

3.1. LC-UV detection

The method of choice for the analysis of catechins in tea has traditionally been reversed-phase LC with UV absorbance detection (Dalluge & Nelson, 2000). Goto et al. (1996) developed a simple and fast high performance method for the analysis of eight tea catechins

Table 2

A summary of the various catechin detection methods and the systems for which they have been used.

Method	System	Reference
<i>LC methods</i>		
HPLC–UV	Human Plasma, Grape seed extract, and green tea extract	Wang et al., 2000; Zuo et al., 2002; Carando et al., 1998; Li et al., 2002
HPLC–DAD	Green tea extract	Bronner & Beecher, 1998; Goto et al., 1996
HPLC–PDA	Green tea extract, oolong tea, black tea, biscuits, and bread	Zuo et al., 2002; Wang & Zhou, 2004; Sharma & Zhou, 2011
HPLC–MS	Coconut water and green tea extract	Chang & Wu, 2011; Flamini, 2003; Dalluge et al., 1997; Poon, 1998; Del Rio et al., 2004
HPLC–MS–electrospray	Green tea extract	Pelillo et al., 2004
HPLC–uorescence	Bulgarian fruits, human plasma, grapes, and red wines	Tsanova-Savova et al., 2005; Gürbüz et al., 2007; Vinas et al., 2000; Carando et al., 1998; Rodriguez-Delgado et al., 2001; Dias et al., 2010
HPLC–FTIR	Green tea infusion	Robb et al., 2002
HPLC–coulometric array detection	Tissues, plasma, and urine	Chu, Wang, Ching, et al., 2004; Chu, Wang, Rogers, et al., 2004
HPLC–electrochemical detection	Green tea extract	Kolouchova-Hanzlikova et al., 2004; Kumamoto et al., 2000; Yang et al., 2000; Lee et al., 2000
HPLC–chemiluminescence	Green tea extract	Nakagawa et al., 1997; Nakagawa & Miyazawa, 1997
Capillary electrophoresis methods		
CE–CZE		Horie et al., 1997
CE–MEKC	Matcha green tea and Green tea extract	Weiss & Anderton, 2003; Bonoli et al., 2003; Aucamp et al., 2000; Nelson et al., 1998; Horie & Kohata, 2000; Larger et al., 1998; Worth et al., 2000; Stach & Schmitz, 2001
CE–ED	Red wine	Peng, Chu, Liu, & Ye, 2004
<i>Other methods</i>		
Thin layer chromatography	Green tea extract and plants	Glavnik et al., 2009; Jerez et al., 2007; Li et al., 1996
Spectrophotometry	Green tea extract	He et al., 2009
FT–NIR spectroscopy	Green tea extract	Chen, Zhao, & Chaitep, 2009
Taste sensor technique	Green tea infusion	Chen et al., 2010
Square wave voltammetry	Green tea extract	Novak, Šeruga, & Komorsky-Lovric, 2010

(i.e. EGCG, EGC, CG, ECG, GCG, C, GC, EC) and caffeine using a water–acetonitrile–phosphoric acid solvent system with two step linear gradients of acetonitrile concentration. All nine chemicals were successfully separated within 20 min. They also found that the temperature of the column oven strongly affected the separation. As the column temperature increased, the retention times of all nine chemicals decreased. Li et al. (2002) developed an HPLC–UV method with gradient elution for the quanti cation of catechins including (+)-catechin, (–)-epicatechin and (–)-epicatechin gallate in grape seed extract. The determination of catechins in human saliva using LC–UV has also been reported (Tsuchiya et al., 1997). Dalluge, Nelson, Thomas, and Sander (1998) noted that a complete separation of catechins and chromatographic quality were column dependent, with endcapped, deactivated, monomeric C₁₈ columns preferable over non-deactivated monomeric or polymeric C₁₈ columns. Also, the presence of acid in mobile phase was essential for both complete resolution of the catechins and efficient chromatography of these compounds. Zuo et al. (2002) developed a simple and fast HPLC method using a PDA detector for simultaneous determination of four major catechins (EGCG, EGC, ECG, and EC), gallic acid and caffeine. After multiple extractions with aqueous methanol and acidic methanol solutions, tea extract was separated within 20 min using a methanol–acetate–water buffer gradient elution system on a C₁₈ column. The PDA acquisition wavelength was set in the range of 200–400 nm. Recently, Wang and Zhou (2004) studied the stability of tea catechins during the breadmaking process. In this study, the catechins were measured using a RP–HPLC system. The separation system consisted of a C18 reversed-phase column, a gradient elution system of water/methanol and formic acid, and a photodiode array UV detector. A similar system was used for the detection of tea catechins in the biscuit matrix (Sharma & Zhou, 2011). In both cases, tea catechins were detected at 275 nm.

3.2. LC–uorescence detection

The use of uorescence detectors has allowed increasing selectivity and sensitivity for the determination of the concentrations of catechins and epicatechins, in addition to other phenolic compounds (Dias, Lovillo, Barraso, & David, 2010). HPLC has been coupled with

uorescence detection to detect catechins in various systems such as Bulgarian fruits (Tsanova-Savova et al., 2005), and red wines and grapes (Dias et al., 2010; Gürbüz et al., 2007). In the case of red wines, a solvent system of methanol–acetic acid–water was used for detection. Linearity was observed in the range of 1 to 30 mg L⁻¹, with limits of detection (LOD) and quanti cation (LOQ) of 0.27 and 0.89 mg L⁻¹, respectively, for catechin and 0.33 and 1.01 mg L⁻¹, respectively, for epicatechin. For Bulgarian fruits, the LOD and LOQ for (+)-catechin and (–)-epicatechin were 0.1 and 0.3 mg/kg fresh weight, respectively. Carando et al. (1998) developed a HPLC method for the determination of (+)-catechin in human plasma, using both uorescence and UV detectors. The LOD and LOQ were 5 ng/mL and 40 ng/mL, respectively. Ultraviolet detection appeared to be less sensitive and selective than uorescence detection. This method provided a simple, accurate, precise and specific method for the determination of (+)-catechin in plasma.

3.3. LC–chemiluminescence/coulometric array detection

Many methods have been reported to measure catechins in tissues. They involve essentially the same technical approaches, using ascorbic acid during the homogenization process and digestive enzymes to release catechins from conjugation. The homogenate is precipitated by water-soluble solvents, such as acetonitrile or ethanol, and extracted by water immiscible solvents, like ethyl acetate, with or without methylene chloride clean-up. After evaporation of the solvents, the residues are subsequently dissolved and analyzed by HPLC with chemiluminescence or coulometric array detection (Chu et al., 2004). Chu, Wang, Ching, et al. (2004), and Chu, Wang, Rogers, Choy, and Pang (2004) developed new methods for simultaneous analyses of eight catechins in tissue, plasma and urine. All eight catechins were analyzed in a single chromatogram within 25 min. The detection limit was 5 ng/mL.

3.4. Capillary electrophoresis detection

Separation and identi cation of the components of green tea have been traditionally performed using liquid chromatography. However, these analyses are typically slow, requiring complex and time



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consuming gradients (Chen, Zhao, Zhiming, & Wang, 2010; Bronner & Beecher, 1998; Dalluge et al., 1998; Dalluge & Nelson, 2000; Weiss & Anderton, 2003) as compared with CE methods. Bonoli et al. (2003) carried out a comparative study between a borate–phosphate–SDS based MEKC and an RP-HPLC method for the separation of seven tea catechins and gallic acid in a green tea extract. Under optimized conditions, HPCE offered several advantage respect to time of analysis (compounds were separated within 4.5 min), sensitivity (HPCE LODs were about 20–100 times lower than HPLC ones) and solvent consumption. HPCE displayed excellent migration time repeatability whereas HPLC showed slightly more quantitation ruggedness (total amount catechins RSD% was 2% for HPLC and 6% for HPCE). CZE (Horie et al., 1997) and MEKC with UV absorbance detection (Nelson et al., 1998) are the CE methods of choice for the determination of catechins. In all instances, uncoated fused-silica capillaries have been used to affect the separations (Dalluge & Nelson, 2000). Early use of CE in the zone mode (Horie et al., 1997) for the separation of catechins in green tea suffered from poor peak resolution. A complementary method for the determination of catechins is the MEKC mode of CE. MEKC offers greater efficiency, selectivity, and speed compared with LC for catechins (Stach & Schmitz, 2001; Weiss & Anderton, 2003). Most of the MEKC methods for the determination of catechins utilize sodium dodecyl sulphate (SDS) micelles in the presence of a borate based running buffer. The MEKC method of Watanabe, Nishiyama, Yamamoto, Nagai, and Terabe (1998) described an impressive separation of seven catechins (C, EC, EGC, ECG, EGCG, GCG, CG), caffeine and ascorbic acid in under 10 min. The resulting MEKC separation was compared to a CZE separation and to an LC separation in terms of overall analysis time and resolving power. In general, the analysis time of the MEKC method (10 min) is shorter than the LC method (20 min) and the resolution by the MEKC method is better than the resolution obtained by CZE. Weiss and Anderton (2003) were the first to report the separation of catechins in matcha using MEKC. Using a 25 mM phosphate buffer at a pH of 7.0 with the addition of 20 mM SDS, detection limits of the seven compounds using the bubble-capillary ranged from 0.1 to 1 µg/mL, which is below the concentrations necessary for analysis of green tea. Recently, Peres, Tonin, Tavares, and Rodriguez-Amaya (2011) developed and validated a sulfated-β-cyclodextrin (s-β-CD) modified reduced flow micellar electrokinetic chromatography (RF-MEKC) method for the determination of catechins in green tea. RF-MEKC has been utilized in cases where it is necessary to reduce the pH to prevent analyte degradation. As a consequence, the electro-osmotic flow (EOF) is significantly suppressed, and a fast anodic migration occurs as the analyte partition into the SDS negatively charged micelles. The optimal electrolyte consisted of 0.2% triethylamine, 50 mmol/L SDS and 0.8% s-β-CD (pH=2.9), allowing baseline separation of five catechins in 4 min. The method demonstrated excellent performance, with limits of detection (LOD) and quantitation (LOQ) of 0.02–0.1 and 0.1–0.5 µg/mL, respectively, and recovery percentages of 94–101%. The method was applied to six samples of Brazilian green tea infusions. Epigallocatechin gallate (23.4–112.4 µg/mL) was the major component, followed by epigallocatechin (18.4–78.9 µg/mL), epicatechin gallate (5.6–29.6 µg/mL), epicatechin (4.6–14.5 µg/mL) and catechin (3.2–8.2 µg/mL).

3.5. Thin layer chromatography detection

TLC is also very useful for rapid screening and quantitation of catechins. To date, TLC detection of catechins has mainly been performed with the aromatic aldehyde vanillin (Jerez et al., 2007) or with *p*-methoxybenzaldehyde (anisaldehyde) (Vovk, Simonovska, Vuorella, & Vuorella, 2002) or 4-dimethylaminocinnamaldehyde (DMACA) (Li et al., 1996). Post-chromatographic derivatization with DMACA is used to enhance sensitivity and selectivity in TLC and HPLC analyses of catechins and proanthocyanidins. Glavnik et al. (2009)

reported the optimization of a sensitive, selective and robust derivatization method using 4-dimethylaminocinnamaldehyde (DMACA) for densitometric determination of (+)-catechin and (–)-epicatechin. The separation of these compounds was achieved by thin-layer chromatography (TLC) on cellulose plates developed with water. The visible limit of detection of both standards was 1 ng, but the densitometric limit of detection was lower (0.2 ng). They found that the optimized DMACA reagent was superior to the more frequently used vanillin reagent and was applicable also for determination of mixtures containing other catechins ((–)-catechin, (–)-epicatechin gallate, (–)-epigallocatechin gallate, procyanidin A2, procyanidin B1 and procyanidin B2).

3.6. Other methods

Several other recent physical and chemical techniques have been applied to the separation and/or quantitation of tea catechins. A brief review of these additional techniques follows.

Arakawa, Kanemitsu, Tajima, and Maeda (2002) developed a highly sensitive analysis methodology to measure catechin employing a peroxalate chemiluminescence detection system. Identification of hydrogen peroxide generated by catechin was determined by ESR as well as peroxalate chemiluminescence using catalase and SOD. As a result, catechin-generated superoxide by electron reduction to dissolved oxygen in basic solution, followed by production of hydrogen peroxide through dismutation reaction. It was possible to measure CC, ECG, EGCG and GA at sensitivities of 10^8 , 10^8 , 10^8 and 10^7 mol/L, respectively. The reproducibility of this method was 2.84–6.1%. The assay time was 30 min for generation of hydrogen peroxide and 21 s for chemiluminescence measurement. This method was also applied to the determination of total catechin levels in green tea, black tea and roasted green tea. Chen et al. (2010) determined the contents of catechins (EGCG, EGC and ECG) and caffeine in green tea using taste sensor technique with multivariate calibration. The system of data acquisitions based on taste sensor was developed in the experiment. Two multivariate calibrations, which were partial least square (PLS) and artificial neural network with principal component analysis (PCA-ANN), were applied to build forecasting models, respectively. Experimental results showed that the PCA-ANN model was superior to the PLS model, and the results of each optimal model were obtained by PCA-ANN as follows: RMSEP (%) = 0.2399, R = 0.9037 for Caffeine model; RMSEP (%) = 0.3101, R = 0.8204 for ECG model; RMSEP (%) = 0.4113, R = 0.8384 for EGC model; and RMSEP (%) = 0.6065, R = 0.9473 for EGCG model. This work demonstrated that taste sensor technique with multivariate calibration can be successfully employed to determine main catechins and caffeine contents in green tea. Robb, Geldart, Seelenbinder, and Brown (2002) investigated the HPLC–FTIR method for determination of catechins and methyl xanthenes in green tea extracts. FTIR detection is suited to the detection of catechin compounds due to their high infrared activity. Furthermore, differences in the infrared spectra of catechins from different classes can be correlated with small structural changes between those classes. A reversed phase separation of the green tea components was performed on a C-18 column equilibrated at 30 °C using an isocratic mobile phase of acetonitrile: 0.1% formic acid (15:85), prior to introduction to the deposition interface linked to the FTIR detector. Six catechins, (+)-catechin, galocatechin, (–)-epicatechin, (–)-epigallocatechin and (–)-epigallocatechin gallate, and (–)-epicatechin gallate, as well as two methyl xanthenes, caffeine and theobromine, were separated and positively identified in a sample of Chinese green tea. With infrared detection, clear structural identification of the different catechins was made and structural differences between catechin classes were identified. (+)-Catechin, galocatechins, and galled catechins, were distinguished, and even subtle structural differences such as (+)-catechin and (–)-epicatechin, which only differ by the *syn/anti* orientation of a hydroxyl substitute, were clearly identified.



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4. Stability of tea catechins during food processing

4.1. Aqueous systems

The stability of tea catechins in aqueous system is dependent on both temperature and pH. Degradation kinetic parameters such as rate constant and activation energy are commonly used to predict the loss of tea catechins during thermal processing in aqueous system (Komatsu et al., 1993; Wang et al., 2006; Zhu et al., 1997; Zimeri & Tong, 1999).

4.1.1. Effects of processing conditions in tea preparation

Wang and Helliwell (2000) reported that tea catechins underwent epimerization at 100 °C which was dominated by epimerization from epistructure to nonepistructure. pH significantly influenced the epimerization rate. More epimerization occurred in tap water (pH 7.1) than purified water (pH 5.9). The complexity of ions in the tap water and the different pH between the tap and purified water were also thought to be the main reasons for the different conversion rates of individual catechins. In the infusion brewed with tap water, the catechins were easily epimerized and then rapidly degraded.

Zimeri and Tong (1999) studied the degradation kinetics of EGCG at different pH (4 to 7) and dissolved oxygen (DO) concentration (0 to 7.59 mg/L) in aqueous system. They validated a mathematical model for EGCG degradation which followed a pseudo-first order kinetic model. Increasing pH and DO concentration resulted in higher rate constant of degradation. However, they did not explore the epimerization mechanism that contributed to this catechin degradation. Wang et al. (2006) proved that both epimerization and degradation of tea catechin in the aqueous system followed pseudo-first-order reactions and the rate constants of reaction kinetics followed the Arrhenius equation. Rate constant and frequency factors were greater in the solution with pH 5.1 than pH 3.5–3.7. The activation energy (E_a) value for the epimerization of EGCG was lower than the epimerization of ECG. However, E_a for the total degradation of EGCG and its epimer GCG was largely the same as that of total ECG and CG.

Komatsu et al. (1993) studied the reaction kinetics of ECG, EC, EGC and EGCG in green tea infusion. They found a turning point of reaction rate constant at 82 °C, below and above which different types of reactions were shown. They suggested that two or more temperature dependent reactions at this point occurred such as epimerization and thermal degradation. However, only the kinetics of an overall reaction was considered for each of the catechins. They also examined that the apparent activation energy above this turning point was 7.3 to 11.4 times higher than that below the turning point which means the reaction rate was much faster above this temperature.

Wang et al. (2008a) conducted a study to re-examine the turning point of 82 °C in the reaction kinetics of brewing tea. Simultaneous epimerization and degradation reactions were considered, all of which were assumed to follow pseudo-first-order reactions. While 82 °C was shown to be nothing unusual, two specific temperature points at 98 and 44 °C were identified. Below 44 °C, degradation reaction was more dominant compared to epimerization reaction. At temperature between 44 and 98 °C, epimerization from non-epicatechin to epicatechin could occur faster, followed by degradation and epimerization from epicatechin to non-epicatechin respectively. Then, above 98 °C epimerization from non-epicatechin to epicatechin was faster than other reactions. These specific points can be considered to modify the temperature profile in brewing tea to get the desired ratio between epimerized and non-epimerized catechins in tea drinks.

Zimmermann and Gleichenhagen (2011) reported that steeping time and temperature of green tea infusion influenced the ingested amount of catechins. Tea leaf infusion at 100 °C for 7 min resulted in three fold increase of flavanols than that at 70 °C for 3 min. Addition of lemon juice before infusion to get pH 3.0 increased 20% of EGCG. They suggested that to get the maximum amount of catechins during

brewing, it was necessary to use boiling water, increase steeping time and add ingredients to reduce the pH.

Labbe, Tremblay, and Bazinet (2006) proposed to classify catechins into two groups according to the way of their changes during brewing. The first group is time dependent catechins (such as EGC and EC) and the other one is time/temperature dependent compounds (EGCG, GCG, ECG). They also introduced a two-step extraction procedure to produce EGC- and EGCG-enriched green tea drinks. These catechins could be controlled since EGC was time dependent and EGCG was time/temperature dependent. They conducted the first step extraction by brewing at 30 °C for 30 min. It aimed to get maximum amount of EGC while most EGCG was still kept in the leaves. Then, the second step extraction was to extract maximum amount of EGCG, which was conducted at 75 °C for 40 min. This temperature avoided a decrease of EGCG which occurred at 90 °C. EGC decreased by 39.0% to 43.9% in the second extraction step. On the other hand, the EGCG content was increased by 41 to 55.5% in the second extraction step.

4.1.2. Effects of sterilization and pasteurization on packaged tea drinks

Bottled or canned ready-to-drink green teas are now preferred by consumers due to their health benefits and convenience. The ingredients and heat treatment used in the production of tea drinks affect the catechins stability. Degradation of catechins also occurs when tea drinks are stored and transported. Therefore, processing, storage and transportation of tea drinks should be of concern for maintaining the stability of green tea catechins.

High temperature heat processing is required to inactivate the spores of thermophilic microbial. Therefore, heat treatment is important in manufacturing canned and bottled tea drinks to extend their shelf life. However, Bazinet, Farias, Doyen, Trudel, and Têtu (2010) reported that heat treatment could lower catechin contents, while other unit operations did not have any impacts. The catechin contents of commercially available tea drinks, except enriched green tea drinks, were very low to provide any health benefits. Therefore, enriched catechins are needed in the tea drinks (Bazinet et al., 2010). Chen et al. (2001) reported that one canned or bottled tea drink (250 mL) contained less green tea catechins (3–60 mg) than one cup of conventional brewed green tea drink (400–500 mg).

Most packaged tea drinks are low acid beverages to maintain catechins stability. During sterilization, epimerization dominated at pH below 5.5, shown by increasing amount of reaction product i.e. the epimer. At pH above 6, the amount of epimer declined, which means both epimerization and oxidation occurred (Komatsu et al., 1993). Autoclaving tea drinks at 120 °C for 20 min decreased the amount of green tea catechins by 24%. Moreover, their stability during autoclaving is pH dependent. They remained stable at pH 3 and 4, but at pH 6 only 20% of green tea catechins remained in the drinks (Chen et al., 2001). The authors also showed that higher temperature increased epimerization of EGCG to GCG. Formation of GCG was the most efficient at pH 5 when EGCG was autoclaved at 120 °C for 20 min.

Controlling the temperature during heat processing is a key to maintain tea catechin stability in ready-to-drink tea products (Kim et al., 2007). Heating conditions have an impact on the epimerization of tea catechins. EGCG, EGC, EC, and ECG undergo epimerization during heating, hence, concentration of total catechins after processing will decline. However, concentration of isomers of these catechins i.e. GCG, GC, C and CG increase as heating temperature increases. Kim et al. (2007) also suggested 85 °C as the temperature for extraction and pasteurization for canned ready-to-drink green tea due to the less changes of catechin concentration at this temperature. Pasteurization at 85 °C reduced EGCG and EGC by 2% and 0.85%, respectively, compared to the heating process at 120 °C which reduced them by 40.22% and 16.67%, respectively. Considering that increasing temperature tended to reduce the total amount of catechins, it was concluded that oxidation occurred during heating



Similarity



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References

besides the epimerization. This oxidation changed the tea liquor color to be darker, less green, and deeper yellow (Kim et al., 2007; Zhu et al., 1997).

Chen et al. (2001) observed that catechins showed varying stability in commercial tea based soft drinks with EGCG and EGC being more unstable than EC and ECG. As reported by some studies, approximately 50% of the tea catechins in the marketed green tea beverages are epimerized by heat treatment (Chen et al., 2001; Kim et al., 2007). The rate of degradation of green tea catechins varies according to the composition of catechin content (Chen et al., 2001; Sang et al., 2005) or to the presence of other compounds such as citric acid or ascorbic acid (Chen et al., 2001; Sang et al., 2005; Wang et al., 2006).

4.1.3. Effects of other ingredients in the production of tea drinks

Sucrose, citric acid and ascorbic acid are commonly used as ingredients in canned and bottled tea drinks. They could either retard or accelerate tea catechin degradation in the tea drinks (Su et al., 2003; Chen et al., 2001). Tea catechins dissolved in sucrose solution (0.15 g/mL, pH 4.0) degraded similarly to tea catechins dissolved in distilled water, while they degraded faster in a solution containing 0.15 g/mL sucrose and 2 mg/mL citric acid (pH 3.02). Ascorbic acid is often used as an antioxidant to prevent tea catechins from oxidative reactions in tea drinks. However, addition of ascorbic acid could maintain the stability of green tea catechins only for one month. In contrast, at longer storage time, ascorbic acid enhanced the degradation of green tea catechins due to its effect as a prooxidant (Chen et al., 2001).

Zimmermann and Gleichenhagen (2011) examined the effect of additives and lowering pH on catechins stability during tea infusion. Addition of citric acid (at pH 3) produced a higher concentration of EGC, EGCG and ECG compared to pure tea. However, addition of ascorbic acid (at pH 6.9 and 4.8) did not affect the amount of catechins. Meanwhile, mixed citric acid and ascorbic acid addition (at pH 3) resulted in a significant different amount of EGC only. It was concluded that ascorbic acid was not effective to reduce catechin degradation which could be achieved by lowering the pH. They also noticed that decreasing pH influenced the structural alteration of catechins, but not the diffusion of catechins into the water. Addition of ascorbic acid to reach pH 3.0 to 6.9 resulted in relatively stable catechins after 3 min steeping time compared to pure tea. These results were due to high concentration gradient between the leaves and the aqueous phase that accelerated diffusion. Also, low concentration of catechins in the aqueous phase slowed down the reaction speed of structural alteration. Furthermore, ascorbic acid could inhibit EGC degradation after 5 min of steeping, but not after 7 min of steeping.

4.2. Lipid and emulsion systems

Lipids make up one of the most essential components of food. They decide not only the taste, scent, color or the texture of a food product, but they also give the feeling of satiety. Edible fats, oils and products including fats undergo the processes of oxidation, both during production and storage. This process in food causes a sequence of unfavorable changes, mainly deterioration in the sensory properties of the product (rancidity, change of color and texture), decrease in nutritional value, increase in the health risk and economic losses (Gramza & Korczak, 2005). Tea catechins act as antioxidants in food delaying free radical accumulation and hence increasing oxidative stability.

Green tea catechins have been known to show varying stabilities in various lipid systems. In the lipid/lipophilic system, initiated by lipid peroxyl radicals, the order of stability was shown to be as ECG = EGCG = EC = C EGC (Salah et al., 1995). In lard, it was shown to be EGCG EGC ECG EC (Madhavi, Singhal, & Kulkarni, 1996), and in Canola oil as EGC EGCG EC ECG (Chen & Chan, 1996). Wanasundara and Shahidi (1996) in their investigations on green tea

catechins antioxidant activity observed that fish oils with catechins showed high oxidative stability in comparison to α -tocopherol, BHT, BHA and TBHQ addition. Antioxidant potentials of catechins were ranked as follows: ECG EGCG EGC EC. The data analysis results supported a hypothesis that the configuration-ortho and number of hydroxyl groups can significantly influence the activity of antioxidants.

In food emulsions the attainment of an adequate structure and the achievement of oxidative and physical stability upon storage represent a challenging goal. The addition of antioxidants is one of the strategies applied to delay lipid oxidation reactions, provided that no sensible modification in the overall properties of the emulsified system occurs (Di Mattia, Sacchetti, Mastrocola, & Pittia, 2009). In emulsions, oxidation is a reaction which is initiated at the interface between the two phases and influenced by both the presence of pro-oxidant and antioxidant compounds and the interactions between the different components of the system (McClements & Decker, 2000). The choice of the antioxidant molecules to add to the system is hence a challenge since the effectiveness of these compounds in emulsion was proven to be very different than in bulk lipids due to the occurrence of interfacial phenomena (Frankel, Huang, Kanner, & German, 1994).

Antioxidants do not show the same activity in different conditions. The antioxidant activity in different lipid systems is affected by their physical state (Frankel et al., 1994, 1996; Huang, Frankel, & German, 1994), different lipid substrates (Hopia, Huang, Schwarz, German, & Frankel, 1996), and pH (Huang, Frankel, Schwarz, Aeschbach, & German, 1996). The methods used to evaluate lipid oxidation also affected the determination of antioxidant activity (Huang et al., 1994). The order of activity and ranking of phenolic antioxidants are dependent on the temperature and other conditions of oxidation (Frankel, 1993). Therefore, to better understand the mechanisms for antioxidant actions, it is important to use different lipid systems and more than one method to evaluate antioxidant activity.

The apparent activity of antioxidants in multiphasic systems depends in part on their effective concentration in the different phases which is, in turn, related to the chemical affinity and the polarity of the molecule. It was suggested that the location of antioxidants and their concentration in different regions of polyphasic systems depends on their polarity and solubility (Huang et al., 1996; Huang & Frankel, 1997; Huang, Frankel, & Lambelet, 1997). Efficiency of antioxidants to a large extent depends on physical state of the lipid-permanent or dispersed phase constituent (McClements & Decker, 2000). It was shown that more unsaturated fatty acids underwent oxidation to a smaller extent in 'oil in water' emulsion than less unsaturated (Miyashita, Nara, & Ota, 1993), while an opposite dependence was noticed in bulk oil (Rosas Romero & Morton, 1975). Frankel et al. (1994) also showed that lipophilic antioxidants had been more effective in emulsion 'oil in water' and the hydrophilic ones in bulk oils.

Being antioxidants, the main role of tea catechins in lipid/emulsion systems is to retard lipid oxidation, extend shelf life of the product and stabilize emulsion systems. However, as shown earlier, the antioxidant activity of different tea catechins can vary depending on the lipid/emulsion system, temperature, pH etc. Huang and Frankel (1997) found that in corn oil-in-water emulsions, all tea catechins were prooxidants at 5 and 20 μ M by accelerating hydroperoxide and hexanal formation. In response to this Almajano, Delgado, and Gordon (2007) stated that catechins were prooxidant at low concentrations but their antioxidant activity was shown at higher concentrations. Roedig-Penman and Gordon (1997), in their study about the antioxidant effects of green tea extracts in model food emulsions, found that components other than EGCG and ECG (for example dimers or other oxidation products formed from EGCG) made major contributions to the antioxidant properties of green tea extracts in stabilizing sunflower oil-in-water emulsions. EGC and EC were expected to be less soluble in water than EGCG or ECG and hence

may be more effective in emulsions due to an increased concentration at the oil–water interface. [Almajano et al. \(2007\)](#) found that model oil-in-water emulsions containing epicatechin (EC) and epigallocatechin gallate (EGCG) showed a synergistic increase in stability in emulsions containing added albumin. The effect of bovine serum albumin (BSA) on model oil-in-water emulsions containing each of the green tea catechins (ECG, EGCG, EC and EGC) was studied during storage at 30 °C. The green tea catechins showed moderate antioxidant activity in the emulsions with the order of activity being ECG = EGCG > EC > EGC. A probable mechanism was proposed for the increase in antioxidant activity of emulsions containing BSA and catechins. It was believed that BSA bound the antioxidant and transported it to the oil–water interface, where it was highly effective at reducing the rate of oxidation. In their study regarding the effect of phenolic antioxidants on the chemical stability of olive oil in water emulsions, [Di Mattia et al. \(2009\)](#) found that catechin showed an interfacial localization which was reflected in the enhancement of primary oxidation and in the inhibition of secondary oxidation. This was due to a rapid inactivation of the peroxy radicals that moved to the interface from the lipidic core and of the radicals formed by hydroperoxide degradation.

4.3. Solid and semi solid systems

4.3.1. Processing of tea leaves

Green tea contains catechins which are approximately up to 75% of total polyphenols. Green tea catechins are relatively stable after each step of green tea processing which includes fresh withered leaf, short withered leaf, pan-fired green leaf, shaped green leaf and red green leaf. These steps could retain the catechins at the range of 21.34% to 24.20% of the tea leaves on a dry-weight basis ([Astill, Birch, Dacombe, Humphrey, & Martin, 2001](#)). EGCG is the major compound in the green tea (7–74 mg/g), followed by ECG (1–41 mg/g), EGC (0–36.5 mg/g), EC (0.1–9.5 mg/g) and C (0–5.8 mg/g) ([Friedman, Levin, Choi, Kozukue, & Kozukue, 2006](#); [Khokhar & Magnusdottir, 2002](#); [Lee & Ong, 2000](#)). [Chen et al. \(2001\)](#) reported that total catechins varied depending on the tea varieties, brands and area of harvest. However, the levels of epicatechins were relatively consistent with EGCG and ECG as the most abundant catechins followed by EC and EGC. Among three kinds of tea, green tea as non fermented product has the highest catechins (8.0–14.4 g/100 g dry tea leaves) followed by oolong tea as a partially fermented product (4.14–4.92 g/100 g dry tea leaves) and black tea (0.24–0.51 g/100 g dry tea leaves) as the effect of oxidation during fermentation ([Chen et al., 2001](#); [Toschi et al., 2000](#)).

During green tea processing, the enzymes which catalyze oxidation are deactivated by heat treatment (pan-roasting or steaming), so that more tea catechins are retained ([Astill et al., 2001](#)). [Friedman et al. \(2009\)](#) conducted a pan frying method in processing of green tea leaves involving roasting, rolling and ring steps to inactivate the enzymes. In the final product, total catechin was reduced by 14.3%. The most abundant tea catechins, i.e. EGCG and ECG showed no significant changes. Meanwhile, the minor catechins, i.e. EGC, GC and EC decreased by 87%, 44%, and 22%, respectively, and C content increased by 78%.

4.3.2. Bakery products

Fortification of green tea in the bakery products has been used to increase their health benefits. Investigation of green tea catechin stability in the breadmaking system was conducted by [Wang, Zhou, and Jiang \(2008b\)](#). They proved that the epimerization and degradation of tea catechin in the breadmaking system followed pseudo first-order reactions and the rate constants of reaction kinetics followed the Arrhenius equation. In the bread system, activation energy (E_a) for epimerization of EGCG to GCG as well as that for GCG to EGCG was similar to those in aqueous solution, which were 105.1 kJ/mol and 84.3 kJ/mol respectively. However, frequency factor (A) for the

epimerization of EGCG to GCG was higher compared to that for GCG to EGCG, and the A value in the crumb higher than that in the crust. Frequency factor depends on the medium pH and the ionization state. Frequency factor in the breadmaking system was higher than that in aqueous system, which might be due to minerals contained in the crumb. Wheat crumb contains considerable amounts of minerals such as iron, calcium, potassium, sodium, magnesium and zinc ([Rosell, 2003](#)), thus altering the stability of tea catechins. During baking, EGCG decreased, while its epimer increased. The catechin stability in the crust was lower than that in the crumb because of its dependence on baking temperature.

[Wang and Zhou \(2004\)](#) showed that green tea catechins underwent epimerization in the breadmaking process. The bread dough was baked at 215 °C for 11 min. The actual core temperature of the dough remained between 80 and 101 °C for 8–9 min, which could provide sufficient energy for catechin epimerization. Also, the pH of bread dough before and after proofing ranged from 5 to 6, under which tea catechins were able to produce their epimers. A similar epimerization was found in biscuits during the biscuit making process ([Sharma & Zhou, 2011](#)). They observed higher percentages of GCG and GGC in the biscuit as compared to their corresponding epimers (ECG and EGCG). It was found that the amount of GCG in the biscuit was more than that added initially in the dough. This could be due to epimerization.

[Lu, Lee, Mau, and Lin \(2010\)](#) substituted 10, 20 and 30% of wheat flour with green tea to make sponge cake and at up to 20% substitution there was no difference of hedonic sensory results. However, substituting 30% green tea did significantly affect the sensory characteristics and was found to be bitter. This substitution enhanced antioxidant activity, reducing power, scavenging ability on DPPH radicals and chelating ability on ferrous ions. After baking in a preheated oven, the concentrations of EGCG and EGC were higher than GCG, ECG, EC as well as catechin. The order of antioxidant properties was reported as follows: EGC = EGCG > ECG > EC > C.

[Sharma and Zhou \(2011\)](#) fortified green tea extract in the biscuits at concentration of 0.15% to 0.3%. They reported that tea catechins were relatively stable in the dough but degraded much after baking due to the alkaline pH of the dough and high temperature during baking at 160 °C. Retentions of ECG and EGCG in the biscuit with 0.3% GTE addition were 29.99% and 21.0% respectively. Catechin stability in the biscuit system was found in a sequence of GCG > GGC > ECG > EGCG.

The stability of green tea catechins in the food systems/products is summarized in [Table 3](#).

5. Stability of tea catechins during storage

[Friedman et al. \(2009\)](#) observed that EGCG decreased by 28% in the tea leaves during storage for 6 months at 20 °C, while ECG reduced by 51%. ECG might be more susceptible to degradation than EGCG because EGCG was more abundant than ECG in the tea leaves. The overall loss of total catechin concentration was 32%. The degradation of EGCG was due to the effect of oxidation process because there was no increase in GCG concurrently with the EGCG reduction ([Friedman et al., 2009](#)).

Table 3
Green tea catechins stability in the food systems/products.

Food systems/products	The order of stability	References
Oil-in-water emulsions	ECG = EGCG > EC > EGC	Almajano et al., 2007
Canola oil	EGC > EGCG > EC > ECG	Huang & Frankel, 1997 Chen & Chan, 1996
Lard	EGCG > ECG > EGC > EC	Madhavi et al., 1996
Fish oil	ECG > EGCG > EGC > EC	Wanasundara & Shahidi, 1996
Dried green tea leaves	EGCG = ECG > EC > GC > EGC	Friedman et al., 2009
Tea drinks	EC = ECG > EGCG = EGC	Chen et al., 2001
Biscuit	GCG > GGC > ECG > EGCG	Sharma & Zhou, 2011
Bread	GC, EC > ECG > GCG, EGCG > EGC	Wang & Zhou, 2004

Chen et al. (2001) conducted a study on canned and bottled tea drinks stored for six months. Dissolving in distilled water, pH 4 buffer and pH 5 buffer led to the degradation of tea catechins by 23%, 55% and 90% respectively. Therefore, it was confirmed that the stability of tea catechins was pH-sensitive. Addition of green tea catechins in a soft drink of pH 3.23 decreased the amount of catechins by 45% after 6 months storage. While, in a soft drink of pH 6, it was completely degraded after 4 months. Sang et al. (2005) showed that the stability of catechins could be extended significantly in a higher catechin concentration liquor. They also reported that there was no significant degradation of catechins during 6 months storage at 4 °C.

Concerning the temperature during storage, Demeule et al. (2002) showed that a lower storage temperature extended appreciably catechin half-life. Frauen, Rode, Steinhart, and Rapp (2000) found that, in cosmetic formulations (oil/water emulsions), catechins decreased to 70% of the initial content at room temperature and only a minimum amount remained at 40 °C after 6 months of storage. Similarly, Spanos, Wrolstad, and Heatherbell (1990) observed a complete degradation of epicatechin and catechin in apple juice after storage at 25 °C for 9 months. The presence and concentration of other ingredients such as sucrose, citric acid and ascorbic acid enhanced the degradation of catechins in a solubilized and purified green tea catechin extract (Su et al., 2003). However, contrary to the above findings, a higher catechin concentration was found to positively extend the shelf life and stability of EGCG and other catechins (Sang et al., 2005). For bakery products, Wang and Zhou (2004) found no change of catechins in bread during 4 days of ambient storage. The same authors also showed that green tea catechins were relatively stable in bread dough during freezing and frozen storage at -20 °C for up to 9 weeks.

Corey, Kerr, Mulligan, and Lavelli (2011) produced freeze dried apple powder with added green tea extract, then stored for 45 days at 30 °C in low and intermediate moisture environments (water activity 0.11 up to 0.75). Apple normally contains substantial amounts of EC, C and ascorbic acid, while the green tea fortified apple contained significant amount of EGCG, EGC, ECG and GCG. They studied the degradation of total monomeric flavan-3-ols which presented total catechins in green tea apple stored at different a_w levels at 30 °C. Degradation of total catechins over 45 days of green tea apple storage followed pseudo first-order kinetic model. The reaction rate constant k of green tea fortified apple was lower than that of dried apple without green tea, suggesting that green tea could help maintain the catechins stability. The value of k increased with increasing a_w .

Ortiz, Ferruzzi, Taylor, and Mauer (2008) showed that storage condition of 43% RH at 22 °C for 3 months maintained the stability of catechins in dried green tea beverage powders. The catechin stability could be retained when the samples were stored below the onset glass transition temperature (T_g). Changes in catechins were affected by sugar, ascorbic acid and citric acid which were all present in apple. Catechins are more susceptible for degradation at higher moisture conditions due to the greater mobility of reactants, dissolution or deliquescence of organic acids. Interactions with food additives impact on the stability of catechins, such as ascorbic acid which has anti-oxidative properties but could change to a pro-oxidant during storage (Zhu, Hammerstone, Lazarus, Schmitz, & Keen, 2003). Corey et al. (2011) showed that catechins degraded more rapidly at higher moisture contents, but not caffeine. The catechin degradation correlated with increasing a_w and T_g , i.e. water mobility. Storage at a_w of 0.75 had the highest impact on catechins stability; the amount of catechins decreased by 39% after the food powder was stored at this condition for 45 days.

6. Conclusion

Epimerization and oxidation were reported as the main cause of changes in tea catechins during food processing and storage. Many factors contributed to the changes. Tea catechin stability is dependent

on pH and temperature. The lower the pH and temperature the more stable the tea catechins are during processing and storage. Tea catechins are stable in acidic system (pH 4); however, in the alkaline system they degrade rapidly. Heat treatments decrease tea catechins due to thermal degradation, oxidation, epimerization and polymerization. In addition, higher oxygen levels and lower concentration of antioxidants increased oxidation of tea catechins. Therefore, avoiding high oxygen exposure during production and storage is important to minimize the autooxidation of catechins that causes quality deterioration of food products. Furthermore, the antioxidative activity of catechins could be increased by the presence of some metal ions. Sucrose, citric acid and ascorbic acid added in the tea drink may either retard or accelerate the catechin degradation in the tea drinks, depending on other conditions. Several detection methods have been developed for tea catechin analysis, which are largely based on liquid chromatography (LC) and capillary electrophoresis (CE) methods for getting a good separation, identification and quantification of catechins. Pseudo first-order kinetic models were developed and validated for the epimerization and degradation of tea catechins, whereas the rate constant of reaction kinetics followed the Arrhenius equation. Heat treatment is the main cause for the degradation of tea catechins during the processing of tea leaves, tea drinks and bakery products. The level of degradations during food processing varies and depends on the initial concentration of tea catechins and other ingredients, pH of the system and processing temperature and duration.

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