Green tea extract: Chemistry, antioxidant properties and food applications – A review

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ABSTRACT

Green tea is one of the most popular and extensively used dietary supplement in the United States. Diverse health claims have made green tea as a trendy ingredient in the growing market for nutraceuticals and functional foods. Green tea extract contains several polyphenolic components with antioxidant properties, but the predominant active components are the flavanol monomers known as catechins, where epigallocatechin-3-gallate and epicatechin-3-gallate are the most effective antioxidant compounds. Additional active components of green tea extract include the other catechins such as epicatechin and epigallocatechin. Among these, epigallocatechin-3-gallate is the most bioactive and the most scrutinized one. Green tea polyphenols are also responsible for distinctive aroma, color and taste. Green tea extract can also be used in lipid-bearing foods to delay lipid oxidation and to enhance the shelf-life of various food products. This review outlines the chemistry, flavour components, antioxidant mechanism, regulatory status, food applications, and stability of green tea extract in food.

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1. Introduction

Tea, derived from *Camellia sinensis* L., is one of the most widely consumed beverages in the world. Tea can be categorized into three main types, depending on the level of oxidation, as green tea, oolong tea and black tea (Chan et al., 2011; Velayutham, Babu, & Liu, 2008). Green tea is an evergreen plant that grows primarily in tropical and temperate regions of Asia which mainly include China, India, Sri Lanka and Japan. It is also cultivated in several African and South-American countries. Two primary varieties of *C. sinensis* are *Camellia sinensis sinensis* and *Camellia sinensis assamica*. The sinensis plant strain is originated from China. This strain produces green, white, black and oolong teas. On the contrary, the assamica plant strain primarily is inhabitant to the Assam region in Northern India. Due to enormous yields of this specific strain, it is the favored plant grown in India, Sri Lanka and some African countries. The leaves of assamica strain are typically used for producing black, oolong and pu’erh teas.

Green tea is a small shrub that can expand up to 30 feet high, but is customarily trimmed to 2–5 feet when cultivated for its tea production. Mature leaves are deeper green in color than immature, light-green leaves are preferably harvested for tea production. The chemical composition of tea leaves has been well documented. The main constituents of tea leaves are polyphenols (Balentine, Wiseman, & Bouwens, 1997). The fresh tea leaves contain caffeine (approximately 3.5% of the total dry weight), theobromine (0.15–0.2%), theophylline (0.02–0.04%) and other methylxanthines, lignin (6.5%), organic acids (1.5%), chlorophyll (0.5%) and other pigments, theanine (4%) and free amino acids (1–5.5%), and numerous flavour compounds (Graham, 1992). In addition, a wide variety of other components exists, including, flavones, phenolic acids and depsides, carbohydrates, alkaloids, minerals, vitamins and enzymes (Chaturvedula & Prakash, 2011). Tea also contains flavonoids, mainly quercetin, kaemferol, myricetin, and their glycosides. The most favorable effects of green tea are accredited to the green tea polyphenols, predominantly the catechins, which make up, 25–35% of the dry weight of green tea leaves (Abdel-Rahman et al., 2011; Balentine et al., 1997; Graham, 1992; Zaveri, 2006). The tea catechins belong to the family of flavonoids (Yilmaz, 2006) and possess two benzene rings referred to as the A- and B-rings. In addition, the catechin molecules contain a dihydroxypyran heterocycle (the C-ring) that has a hydroxyl group on carbon 3. Moreover, the A-ring is similar to a resorcinol moiety whereas the B-ring is similar to a catechol moiety. The catechin molecule has two chiral centers on carbons 2 and 3. Hence, it has four diastereoisomers with two of the isomers are in trans configuration, and the other two are in cis configuration. The trans and cis isomers are referred to as the catechin and epicatechin, respectively. These chemical structures appear to be important for the antioxidant activities of tea polyphenols, including an ortho-3'-4'-dihydroxy group or 3’4’-trihydroxyl group in the B-ring, a gallate group located at the 3 position of the C-ring, and hydroxyl groups at the 5 and 7 positions of the A-ring (Rice-Evans, Miller, & Paggana, 1996). Many structure–activity relationship investigations have been performed on the antioxidant activity of flavonoids, including tea catechins (Farkas, Jakus, & Héberger, 2004; Guo et al., 1999; Harborne & Williams, 2000; Heim, Tagliaferro, & Bobilya, 2002; Rice-Evans et al., 1996). According to these studies, the antioxidant activity of flavonoids depends substantially on the number and position of hydroxyl groups in the molecule (Farkas et al., 2004). In addition, several structural elements such as o-dihydroxyl catechol structure in the B-ring, the presence of unsaturation and 4-oxo group in the C-ring are also presumed to increase the antioxidant activity of flavonoids. The 2,3-double bond in the C-ring along with 4-oxo function in the C-ring facilitates electron delocalization from the B-ring. Moreover, hydroxyl groups at positions 3 and 5 providing hydrogen bonding to the 4-oxo group in the C-ring is another structural feature attributed to the antioxidant activity of flavonoids.

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2. Chemistry

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Fig. 1 – Chemical structures of major catechins found in green tea extract.

Extracts of immature tea leaves are rich in flavanols and their gallic acid derivatives, namely, (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, and (-)-epigallocatechin gallate (Wanasundara & Shahidi, 2005). In addition, they contain a range of natural green tea flavour components such as terpenes, sesquiterpenes, and organic acids. As exemplified in Fig. 1, catechins are characterized by various hydroxyl groups on the A- and B-rings (Velayutham et al., 2008). Epicatechin has an ortho-dihydroxy group in the B-ring at carbons 3’ and 4’ and a hydroxyl group at carbon 3 on the C-ring (Fig. 1). Epigallocatechin differs from epicatechin in that it has a trihydroxy group at carbons 3’, 4’, and 5’ on the B-ring (Yilmaz, 2006). Epigallocatechin gallate differs from epicatechin in its gallate moiety positioned at carbon 3 of the C-ring (Yilmaz, 2006). However, epigallocatechin gallate is composed of trihydroxy groups on the B-ring and a gallate moiety located at carbon 3 of the C-ring (Yilmaz, 2006). In epigallocatechin gallate, these trihydroxy groups are located at carbons 3’, 4’ and 5’ on the B-ring. Yilmaz (2006) has reviewed the chemistry and application perspectives of green tea, especially in relation to using their catechin components. The relative content of green tea catechins depends on how the leaves are processed prior to drying. Fermentation and heating of tea leaves during the manufacturing process may cause polymerization of monophenolic catechins, leading to conformational changes and thus modifying their properties (Cabrera, Artacho, & Giménez, 2006).

Green tea contains significantly higher amounts of polyphenols as compared to black or oolong tea, owing to differences in the processing of tea leaves following harvest. For green tea, freshly harvested tea leaves are steamed at high temperatures and dried to deactivate the polyphenol oxidases and, as a result, preventing the oxidation of catechins and maintaining the polyphenols in their monomeric forms. In addition to preventing polyphenol oxidation, the steaming process also protects against enzymatic degradation of vitamins. Through separation, purification, concentration and the drying processes, highly concentrated green tea catechin extract with low flavour intensity can be produced. Black tea, on the other hand, is produced by extended fermentation of tea leaves, which results in, the development of polymeric compounds including thearubigin and theaflavins (Zaveri, 2006). Black tea contains predominantly gallates of epicatechin. Oolong tea, a partially fermented product, contains a mixture of the monomeric polyphenols and high-molecular-weight theaflavins (Graham, 1992; Zaveri, 2006). All three varieties of tea contain significant quantities of caffeine (3–6%) which is unaffected by different processing conditions (Chu, 1997; Zaveri, 2006). Furthermore, all three varieties of tea contain (–)-epicatechin, (–)-epigallocatechin, (–)-epicatechin gallate and (–)-epigallocatechin gallate, with the exception of catechin (Khokhar, Venema, Hollman, Dekker, & Jonge, 1997). Epicatechin gallate and epigallocatechin gallate are considered to be the main catechins found in black tea (Obanda, Owuor, & Mang’oka, 2001; Yilmaz, 2006). Epigallocatechin gallate is the most copious catechin component present in the leaves of green, black and oolong teas (Graham, 1992). In general, the contents of epigallocatechin gallate in green and oolong teas range from 22 to 53 mg/g of commercial teas (Zuo, Chen, & Deng, 2002). However, epigallocatechin gallate content in black teas is about 4.0 mg/g of commercial teas (Zuo et al., 2002).

The stability of green tea catechins depends on the pH and temperature. In acidic solutions (pH < 4), green tea catechins exhibit exceptional stability; in alkaline solutions (pH > 8), however, they are extremely unstable (Zhu, Zhang, Tsang, Huang, & Chen, 1997). The study conducted by Zhu et al. (1997) demonstrated that (–)-epigallocatechin gallate and (–)-epigallocatechin were more unstable than (–)-epicatechin and (–)-epicatechin gallate in a basic solution, giving a plausible explanation to the fact that partial absorption of green tea catechins in red blood cells of mice is attributed to the instability of (–)-epigallocatechin gallate and (–)-epigallocatechin in the intestine where the pH is neutral or alkaline. In a high temperature environment, green tea catechins are not very stable. Heating may cause the conversion of green tea catechins to their corresponding isomers, a process known as epimerization. For example, as epimerization can occur at high temperature, epigallocatechin gallate in green tea extract may convert to its epimer component gallatechin gallate. Heat treatments decrease the antioxidant activity of green tea catechins due to oxidation, thermal degradation, epimerization and polymerization (Ananingsih, Sharma, & Zhou, 2013).

3. Flavour constituents

The taste and flavour of tea is governed by key chemical components which are polyphenols, caffeine, organic acids and volatile terpenes (Borse, Rao, Nagalakshmi, & Krishnamurthy, 2002). The characteristic taste of green tea extract is made up of a mixture of bitterness, astringency, meaty (“umami”) taste, sweetness and slight sourness. The compounds, which contribute to, characteristic tea taste, are polyphenolic compounds, amino acids and caffeine (Yamanishi, 1995). Volatile compounds such as terpenoids, alcohols and carbonyl compounds contribute to the aroma of tea. Volatile fractions of various green teas reported having numerous active aroma compounds which are responsible for nutty, floral, fruity,
meaty, popcorn-like, metallic, potato, green, cucumber-like, and hay-like characteristics (Kumazawa & Masuda, 2002). In a study conducted by Pripdeevech and Machan (2011), the volatile flavour components of different teas growing in Thailand were evaluated. These authors reported that the utmost volatile flavour components in green tea were found to be hotrienol, geraniol and linalool. In addition, green Assam tea was reported to contain linalool, geraniol and α-terpineol as the key flavour constituents (Prdeepveech & Machan, 2011).

In a classic study to understand the taste characteristics of different types of catechins, Nakagawa (1979) found that (+)-catechin, (−)-epicatechin, and (−)-epigallocatechin were bitter with a sweet aftertaste while (−)-epicatechin gallate and (−)-epigallocatechin gallate were bitter and astringent. In a follow up study to determine the chemical components that affect the taste attributes of green tea in Japan, Nakagawa (1979) discovered that teas containing higher content of catechins and amino acids scored much higher in taste tests by consumers. His results indicated that the astringency and bitterness of green tea was primarily determined by the content of catechins and other phenolic compounds. The taste of oolong tea appears to be unique. According to Chaturvedula and Prakash (2011), the mellowness and sweetness of oolong tea are attributed to non-oxidized catechins, thearubigins, some secondary polyphenolic compounds, caffeine, free amino acids and related sugars. The astringency of oolong tea is lower, and the sweet taste is stronger than those of green tea. The distinctive taste of black tea is due to the combination of several attributes such as bitterness, astringency, sweetness, malty taste, green/grassy taste, ‘caramel-like’ and ‘hay-like’ characterstics (Alasalvar et al., 2012). Yang and Landau (2000) reported that the theaflavins are crucial to the characteristic color and taste of black tea. The theaflavins contain numerous compounds including, theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate and theaflavin-3,3′-digallate (Chan et al., 2011). The key astringent taste compounds in black tea are catechins, theaflavins, and flavonol glycosides (Scharbert, Holzmann, & Hofmann, 2004). Although compounds in all three categories are detected as astringent, flavonol glycosides are the most abundant contributors of astringency in black tea (Scharbert et al., 2004).

Amino acids are the components in green tea that contribute full-bodied umami flavour and sweetness. Of these amino acids, more than 50% are theanine, which is unique to tea (Chaturvedula & Prakash, 2011). The amino acid theanine has a chemical structure somewhat similar to that of glutamine, with its distinct attribute being a refined, rich flavour and sweetness. Amino acids other than theanine present in tea leaves are glutamine, alanine, asparagine, arginine and serine. The umami taste of green tea is shown to be due to theanine and serine. It is believed that 70% of the umami taste in Japanese green tea is due to theanine (Kaneko, Kumazawa, Masuda, Henze, & Hofmann, 2006). By conducting molecular and sensory studies, Kaneko et al. (2006) concluded that theanine, gallic acid, theogallin and succinic acid are the main compounds responsible for umami taste of green tea. Besides catechins and caffeine, some amino acids, in particular, arginine and alanine also contribute to the bitterness of green tea (Chaturvedula & Prakash, 2011).

4. Antioxidant mechanism

Lipid oxidation and development of rancidity is a major challenge for food manufacturers, reducing shelf-life and altering the quality and nutritional value of their products. Autoxidation, the most common process leading to oxidative deterioration of food lipids, is a free radical chain reaction that proceeds through three distinct stages of initiation, propagation and termination. The generation of primary free radicals is facilitated by the presence of oxidation initiators such as light, heat, ionizing radiation, transition metals, metalloproteins, oxidants, various homolysis-prone substances and enzymes. Lipid hydroperoxides have been identified as primary products of autoxidation. Typically, lipid hydroperoxides are tasteless and odorless. The decomposition of hydroperoxides yields aldehydes, ketones, alcohols, hydrocarbons and acids, which are known as secondary oxidation products of lipids. In many cases, these compounds are responsible for off-flavours and off-odors in food. Antioxidants are of interest to the food industry because they can delay development of oxidative rancidity in food. An antioxidant is a substance that retards lipid oxidation either by inhibiting initial free radical formation or by preventing them from producing more free radicals which can disseminate the oxidation reaction. These substances help preserve foods by delaying development of rancidity, deterioration and discoloration due to lipid oxidation. There are two main categories of antioxidants in relation to their mechanism of action: primary antioxidants and secondary or preventive antioxidants. Primary antioxidants disrupt the oxidative free radical chain reaction by donating electrons or hydrogen atoms from the phenolic hydroxyl groups and, therefore, stabilize lipid free radicals, as a result, inhibit or slow down the initiation phase and disrupt the propagation stage of autoxidation. Secondary antioxidants deactivate singlet oxygen, chelate metal ions (i.e., iron, copper), absorb ultraviolet radiation, scavenge oxygen and help regenerate primary antioxidants. For better effectiveness, primary antioxidants are often used in combination with secondary antioxidants.

Tea polyphenols, mainly flavonoids, are well-known for their antioxidant properties. The antioxidant activity of green tea polyphenols is primarily attributed to the combination of aromatic rings and hydroxyl groups that assemble their chemical structure and consequently binding and neutralization of lipid free radicals by these hydroxyl groups. Numerous studies have demonstrated that polyphenols and tea catechins are exceptional electron donors and effective scavengers of physiologically relevant reactive oxygen species in vitro, including superoxide anions (Guo et al., 1999; Michalak, 2006; Nakagawa & Yokozawa, 2002; Nanjo et al., 1993; Velayutham et al., 2008), peroxyl radicals, and singlet oxygen (Guo et al., 1999; Michalak, 2006). Catechins also exhibit antioxidant activity via chelating redox active transition-metal ions. Polyphenolic compounds, possess hydroxyl and carboxyl groups, are able to bind particularly iron and copper (Michalak, 2006). There is another mechanism underlying the antioxidant ability of plant polyphenols, including tea catechins. Transition metal ions typically have the ability to initiate free radical chain oxidations by decomposing lipid
hydroperoxides (LOOH) by the hemolytic cleavage of the O–O bond and producing lipid alkoxyl radicals. Phenolic antioxidants, including tea catechins, inhibit lipid peroxidation by binding these lipid alkoxyl radicals. This activity depends on the structure of the molecules, and the number and position of the hydroxyl group in the molecules (Milič, Djljas, & Canadanovic-Brunet, 1998). Green tea catechins also exhibit antioxidant activity through inhibiting pro-oxidant enzymes and inducing antioxidant enzymes (Velayutham et al., 2008).

The antioxidant activity of these catechins and the derivatives showed a marked difference depending on the substrate used for evaluation (Wanasundara & Shahidi, 2005). Green tea catechins, which performed like other hydrophilic antioxidants such as Trolox (a water-soluble analog of \( \alpha \)-tocopherol) and ascorbic acid, have been shown to be active antioxidants in bulk oils, but were prooxidants in the corresponding oil-in-water emulsions (Frankel, Huang, & Aeschenbach, 1997; Frankel, Huang, Kanner, & German, 1994). In corn oil triacylglycerols system that was oxidized at 50 °C the antioxidant activities of epigallocatechin, epigallocatechin gallate and epicatechin gallate were superior to those of epicatechin or catechin (Huang & Frankel, 1997). These catechins have also been highly successful in delaying oxidation of polyunsaturated fatty acids-rich marine and vegetable oils (Wanasundara & Shahidi, 1998). In the lecithin-containing liposomes system, epigallocatechin gallate was superior to other compounds such as epicatechin, epigallocatechin, epicatechin gallate, and catechin (Huang & Frankel, 1997). In the oil-in-water emulsions, however, all catechin compounds demonstrated pro-oxidant activity (Huang & Frankel, 1997). The improved antioxidant activity observed for tea catechins in liposomes compared to emulsions has been explained by the greater affinity of the polar catechins toward the polar surface of the lecithin bilayers, thus affording better protection (Huang & Frankel, 1997). In a study conducted by Zhong and Shahidi (2011), epigallocatechin gallate was structurally modified to improve its lipophilicity via esterification of epigallocatechin gallate with selected fatty acids, such as stearic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The lipophilized derivatives so produced exhibited greater antioxidant activity than the original epigallocatechin gallate molecule.

Several investigators have speculated the mechanism of antioxidant action of (+)-catechin using some oxidation model studies (Hirose, Yamamoto, & Nakayama, 1990; Koketsu, 1997; Zhu et al., 2000). As indicated by the proposed mechanism, (+)-catechin molecule can scavenge four lipid free radicals per molecule (Hirose et al., 1990; Koketsu, 1997). The antioxidant activity of individual tea polyphenols in different model systems showed a proportional relationship to the number of hydrogen radical donors of catechins. A synergistic effect has been observed between green tea catechins and ascorbic acid and \( \alpha \)-tocopherols (Murakami, Yamaguchi, Takamura, & Matoba, 2003). Catechin, a monomeric flavanol, is reported to have hydroxyl, peroxyl, superoxide and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities (Bors & Michel, 1999; Fukushima & Mazza, 2000). Moreover, tea catechins have the ability to chelate iron in food model systems (Tang, Berry, Sheehan, & Buckley, 2002). Nakao, Takio, and Ono (1998) reported that the peroxyl radical scavenging activities of catechin, epicatechin and epicatechin gallate were ten times higher than those of \( \alpha \)-ascorbate and beta-carotene. In another study, Nanjo et al. (1996) documented that DPPH radical scavenging activities of catechin and epicatechin are less than epigallocatechin, epicatechin gallate, and epigallocatechin gallate. As expected, epicatechin is another monomeric flavanol (Yilmaz, 2006) came across in green tea. Reports have shown that epicatechin is capable of scavenging hydroxyl radicals, peroxyl radicals, superoxide radicals, and DPPH radicals (Bors & Michel, 1999; Fukushima & Mazza, 2000; Liu, Ma, Zhou, Yang, & Liu, 2000; Yilmaz, 2006). Yashin, Yashin, and Nemzer (2006) examined the antioxidant activity of different types of teas that include green, oolong, black and pu-erh teas. The antioxidant activity of teas descends in the following order: green > oolong > black > pu-erh tea. Chan et al. (2011) evaluated the role of non-polymeric phenolic (NP) and polymeric tannin (PT) constituents in the antioxidant and antibacterial properties of six brands of green, black, and herbal teas. Extraction yields of the products ranged from 12% to 23%. Much higher fractionation yields more NP constituents (70-81%) than PT constituents (1-11%), which suggested that, the former are the principal tea components. In general, the antioxidant properties of green tea extracts were stronger than those of black and herbal teas (Chan et al., 2011). For all six brands tested, antioxidant properties of PT fractions were significantly higher than crude extracts and NP fractions. Although PT constituents have stronger antioxidant and antibacterial properties, they constitute only a minor component of the teas (Chan et al., 2011).

Green tea polyphenols have been shown to be effective against beta-carotene oxidation. For example, tea catechins have been able to demonstrate an anti-discoloring effect on beverages and margarine containing beta-carotene (Koketsu, 1997; Unten, Koketsu, & Kim, 1997). It was suggested that the discoloration of beta-carotene and the oxidation of unsaturated fatty acids progressed by the same mechanism. In particular, carbon–carbon double bonds in beta-carotene and unsaturated fatty acids are attacked by radicals, becoming hydroperoxides. Green tea catechins have the ability to suppress the degradation of double bonds as radical scavengers. It was also suggested that the hydroxyl group at the 5′-position of the B-ring of the catechin structure mostly contributed to the anti-discoloring effect. Hence, as described above, it was speculated that green tea polyphenols delayed the degradation of beta-carotene by acting as antioxidants following the same mechanism. Among the individual green tea polyphenols examined, gallocatechin gallate, epigallocatechin gallate, epigallocatechin, and gallo catechin showed strong anti-discoloring effect while epicatechin and catechin showed almost no activity, and gallic acid had moderate activity (Unten et al., 1997).

5. Regulatory status

In the United States, green tea extract may be used as flavour agent with antioxidative properties in various fats, oils and foods containing fats and oils. Green tea has a history of safe use as food flavouring and is known for its antioxidative functionality as a secondary effect. Green tea extract is well known in Asian food. According to the United States Code...
Proper incorporation of green tea extract into food products is imperative to ensure that antioxidant components are thoroughly dissolved and/or homogeneously dispersed in the food matrix. As only a small amount of green tea extract may be required for adequate shelf-life extension in food, the method of application may determine the achievement of the antioxidant effectiveness. The selection of application methods may depend on the type of food products, processing methods, and the availability of the equipment used for food processing. Commercial extracts of green tea are mainly regulated as an antioxidant and must be specifically applied in certain formulations containing dry ingredients as this enables easier blending. The powder carrier aids the incorporation and homogeneous distribution of the green tea extract. Where dry ingredients are part of the formulation, a powdered green tea extract is often preferred due to easier blending. Conversely, Green tea extract can be dissolved in a food-grade solvent to produce an oil soluble or dispersible liquid product. Liquid forms of green tea extracts may be added to food products by direct addition into oils and fats. The fat or oil may be heated to an elevated temperature (i.e., 40–60 °C) while stirring. Liquid green tea extract should then be added slowly into melted fat or oil. After addition is completed, agitation should be continued for an additional period of time for complete and uniform distribution of green tea extract in fats and oils.

6. Method of incorporation into food

Proper incorporation of green tea extract into food products is imperative to ensure that antioxidant components are thoroughly dissolved and/or homogeneously dispersed in the food matrix. As only a small amount of green tea extract may be required for adequate shelf-life extension in food, the method of application may determine the achievement of the antioxidant effectiveness. The selection of application methods may depend on the type of food products, processing methods, and the availability of the equipment used for food processing. Commercial extracts of green tea are mainly available in a fine-grained powder form. Green tea extract can be solubilized in water prior to addition into foods. Water-soluble green tea extract has a low viscosity for easy spraying and homogeneous distribution. Moreover, remarkably little energy is required to produce the dispersion in water. On the other hand, green tea extract in powder form is often-preferred in certain formulations containing dry ingredients as this enables easier blending. The powder carrier aids the incorporation and homogeneous distribution of the green tea extract. Where dry ingredients are part of the formulation, a powdered green tea extract is often preferred due to easier blending. Conversely, Green tea extract can be dissolved in a food-grade solvent to produce an oil soluble or dispersible liquid product. Liquid forms of green tea extracts may be added to food products by direct addition into oils and fats. The fat or oil may be heated to an elevated temperature (i.e., 40–60 °C) while stirring. Liquid green tea extract should then be added slowly into melted fat or oil. After addition is completed, agitation should be continued for an additional period of time for complete and uniform distribution of green tea extract in fats and oils.

7. Application in food products

There is growing consumer interest in the green tea extract-supplemented products in recent years. Green tea extract has been used in a variety of food products including bread (Wang & Zhou, 2004), biscuits, dehydrated apple (Lavelli, Vantaggi, Corey, & Kerr, 2010) and various meat products (Mitchum, O’Grady, Kerr, & Buckley, 2005; O’Sullivan, Lynch, Lynch, Buckley, & Kerr, 2004; Tang, Kerry, Sheehan, & Buckley, 2001). The main application areas of green tea extract are summarized in Table 1. In internal tests with fat spreads, green tea extract showed comparable antioxidant performance to conventional synthetic antioxidant tert-butyldihydroquinone (TBHQ) and may be more cost-effective than other natural sources of antioxidants. This makes it ideal for both full-fat margarines as well as oxidation-sensitive products that are low in fat, free of trans fatty acids or have a high content of polyunsaturated fatty acids. In this study, samples of 40% low-fat spread were produced with green tea extract containing 60 ppm total catechins or 100 ppm TBHQ and stored at 5 °C for 13 weeks. A control sample contained no antioxidant solution. In an accelerated shelf-life test, a comparison of green tea extract and TBHQ revealed similar performance.

Lipid oxidation is one of the primary causes of quality deterioration in meat and poultry products. Red meats and poultry are typically highly susceptible to lipid oxidation due to their high fat content (Yilmaz, 2006) and a high proportion of polyunsaturated fatty acids. These highly unsaturated phospholipids present in meat and poultry products are responsible for the development of off-flavours and off-odors after cooking and subsequent refrigerated storage. This off-flavour development, also referred to as warmed-over-flavour (WOF), frequently causes consumers to reject freshly cooked meat and poultry products. Therefore, these products should be protected by natural sources of antioxidants such as green tea extract to protect against WOF.

In a study conducted by DuPont Nutrition & Health laboratories, both control and green tea extract-stabilized turkey burgers were made using standard industry practice. Green tea extract was added, at a dosage level of 50-100 ppm. Samples were evaluated for thiobarbituric acid reactive substances (TBARS) formation, reported as malondialdehyde (a secondary product of lipid oxidation) equivalents. Results
showed that oxidation in roasted turkey burgers was significantly retarded using green tea extract, at levels of 50 to 100 ppm. Samples supplemented with green tea extract had lower levels of TBARS during storage as compared to control with nothing added (Fig. 2). In another study conducted by DuPont Nutrition & Health, the efficacy of green tea extract was compared with equivalent dosages of butylated hydroxyanisole (BHA) and revealed that a dosage of 200 ppm of green tea extract simply provided the same antioxidant protection as 40 ppm BHA in chilled (4 °C) roasted turkey burgers during storage (Fig. 3). The sensory evaluation of the products further confirmed the advantage of using green tea extract compared to BHA. In a different study conducted by DuPont Nutrition & Health, the addition of 150 ppm green tea extract was adequate to inhibit hexanal development (a secondary product of lipid oxidation) during 13 days of storage in roasted beef burgers (Fig. 4). This study compared the antioxidant capacity obtained from 150 ppm green tea extract with that obtained from 375 ppm rosemary extract. Green tea and rosemary extract-supplemented samples had low levels of hexanal throughout storage when compared with the control containing nothing added. Furthermore, green tea extract-supplemented sample had lower levels of hexanal as compared to the rosemary extract-treated sample throughout storage (Fig. 4).

The inhibitory effect of green tea extract on the oxidation of meat lipids was also shown by other investigators. Studies conducted by Tang et al. (2001) and Mitsumoto, O’Grady, Kerry, and Buckley (2005) showed a high antioxidant activity of green tea catechins in beef and chicken meat. In another study, O’Sullivan, Lynch, Lynch, Buckley, and Kerry (2004) evaluated the effect of several natural extracts on oxidative stability of chicken nuggets during refrigerated (4 °C) storage. Nuggets were supplemented with rosemary extract (at

Table 1 – Prospective applications of green tea extract.

<table>
<thead>
<tr>
<th>Application</th>
<th>Specific oxidation issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw meats</td>
<td>Oxidation of red meat pigments, resulting in an undesirable brown color</td>
</tr>
<tr>
<td>Cooked meat, poultry and seafood products</td>
<td>Susceptible to oxidation, resulting in a warmed-over flavor, discoloration and protein degeneration</td>
</tr>
<tr>
<td>Ready-to-eat meals</td>
<td>Reheating of meat promotes the oxidation process</td>
</tr>
<tr>
<td>Cereals, bakery products, confectioneries,</td>
<td>Products are susceptible to oxidation due to long shelf-life requirements</td>
</tr>
<tr>
<td>snack foods, nuts and nut products</td>
<td></td>
</tr>
<tr>
<td>Oil-in-water emulsions</td>
<td>Large oil–water interface and complex food matrix increase susceptibility to lipid oxidation</td>
</tr>
<tr>
<td>(mayonnaise, salad dressings, soups and sauces)</td>
<td>Large water–oil interface and complex food matrix increase susceptibility to lipid oxidation</td>
</tr>
<tr>
<td>Water-in-oil emulsions</td>
<td>Low oxidative stability, due to trans fatty acid regulations, increases the need for enhanced antioxidant protection</td>
</tr>
<tr>
<td>(margarine and fat spreads)</td>
<td>Products are susceptible to oxidation due to long shelf-life requirements</td>
</tr>
<tr>
<td>Vegetable oils, marine oils, frying oils, and</td>
<td></td>
</tr>
<tr>
<td>shortenings</td>
<td></td>
</tr>
<tr>
<td>Beverages (carbonated and non-carbonated</td>
<td></td>
</tr>
<tr>
<td>beverages, energy drinks, soft drinks, and</td>
<td></td>
</tr>
<tr>
<td>juices)</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2** – TBARS of roasted turkey burgers during storage at 4 °C.

**Fig. 3** – Effect of green tea extract and BHA on the inhibition of TBARS of roasted turkey burgers during storage at 4 °C.

**Fig. 4** – Hexanal contents of roasted beef burgers during storage at 4 °C.

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1000 ppm), sage extract (at 1000 ppm), or tea catechins (at 100 ppm). Their results indicated that the addition of natural extracts, including tea catechins, reduced lipid oxidation in chicken nuggets both in the presence and absence of salt. However, when sodium tripolyphosphate (STPP) was incorporated into the same product system, the efficacy of natural extracts was significantly reduced.

In addition to providing antioxidant activity and natural flavor attributes, green tea extract can also help improve the color stability of fresh meat products. Fresh meat color depends on myoglobin that stores oxygen for aerobic metabolism in the muscle. Iron is a pivotal player in meat color. One of the defining factors of meat color is the chemical state of iron. Oxymyoglobin gives meats the red color. The brownish color is due to metmyoglobin formed by oxidation of oxymyoglobin. However, how green tea extract influences the color cycle in meat products is not well understood. Studies conducted by DuPont Nutrition & Health have shown that the red color of raw ground beef patties (20% fat) was significantly improved by the addition of rosemary extract (1000 ppm) and green tea extract (250 ppm) compared to the development of browning in control samples (Fig. 5). All samples were stored at 0–2°C for 22 days and packaged under modified atmosphere (80% oxygen and 20% carbon dioxide) packaging conditions. Determination of redness of the meat (‘a’ value using Minolta Colorimeter) corresponded with the development as the visible red color. The meat had lost almost if not all visible red color when the ‘a’ value reached approximately 12. The control reached this point around day 9, and the treated samples around day 16. This shows that 1000 ppm rosemary extract and 250 ppm green tea extract exhibited good protection of color for several days beyond the control sample, and there was a minimal difference between the two treatments (Fig. 5). Sensory evaluation of the burgers showed that green tea extract had no unfavorable flavor impact on the finished burgers where slight rosemary flavor could be detected in the samples containing rosemary extract. In addition, green tea extract contributed to “meaty” (umami) taste in finished burgers (data not shown). In flavour sensitive meat systems, green tea extract would be a more viable option than the rosemary extract due to similar effectiveness, but lower flavour impact. A study by Moawad, Abouzeid, and Nadir (2012) has shown that the use of tea catechins in combination with nitrite was more effective in keeping quality of dry-cured sausages during refrigerated storage as indicated by attractive red color (a* value) as compared to samples treated with nitrite. In another study conducted by DuPont Nutrition & Health laboratories, cured ham slices containing 500 ppm sodium ascorbate and 300 ppm green tea extract enhanced color stability (from 14 days to 25 days) by maintaining the redness remarkably efficiently as compared to a standard ham with 500 ppm sodium ascorbate. The ham slices were packaged under MAP conditions (80% oxygen and 20% carbon dioxide) and stored at 5°C for a period of 38 days. This was shown visually and measured by a VideometerLab 2 – High Performance Multispectral Imaging by recording a-values. The a-value of 17 was defined as the working limit for redness acceptability of cured ham.
components. Consequently, in follow-up studies, a column chromatography was utilized to eliminate chlorophyll compounds from green tea extracts. The dechlorophyllized green tea extract so produced was incorporated into both seal blubber and menhaden oils at various levels. The antioxidative activity of dechlorophyllized green tea extract was compared with that of the butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), TBHQ and \( \alpha \)-tocopherol. At the levels of \( \geq 200 \) ppm, dechlorophyllized green tea extract showed exceptional antioxidative activity in both oils examined. Moreover, the efficacy of dechlorophyllized green tea extract was superior to that of the BHA (at 200 ppm), BHT (at 200 ppm) and \( \alpha \)-tocopherol (at 500 ppm), but inferior to that of TBHQ (at 200 ppm). In several other studies, antioxidant activity of tea polyphenols in vegetable oils and animal fats was examined (Chen et al., 1996; Koketsu, 1997). Crude green tea extract powders were more effective than \( \alpha \)-tocopherol and BHA in lard under conditions of the active oxygen method (AOM) at 97.8 °C (Matsuzaki & Hara, 1985). In a \( \beta \)-carotene-linoleate model system, \((-\)-epicatechin-3-gallate exhibited the strongest antioxidative activity whereas \((-\)-epigallocatechin had the weakest performance (Amarowicz & Shahidi, 1995). Furthermore, the antioxidative efficacy of \((-\)-epicatechin and \((-\)-epigallocatechin-3-gallate was similar and in between those of \((-\)-epicatechin-3-gallate and \((-\)-epigallocatechin. In another study, Milder-Szkudlarz et al. (2009) examined the feasibility of using green tea extract to improve the oxidative stability of biscuits during storage. The antioxidative activity of green tea extract was compared with commonly used synthetic antioxidant BHA. Phenolic compounds of green tea extract had significantly higher DPPH radical scavenging activity than BHA. However, both green tea extract and BHA were effective inhibitors of the decomposition of hydroperoxides. Moreover, biscuits treated with green tea extract had better sensory profile during storage as compared to samples containing BHA.

8. Stability tests

Oxidative stability is a decisive factor in the processing and marketing of oils, fats, and lipid-bearing foods. Methods for determining oxidative stability are, therefore, essential particularly when green tea extract is being evaluated for its effectiveness in delaying lipid oxidation in these products. Consequently, many protocols have been developed in attempts to assess the effectiveness of green tea extract in food. Oxidative stability of green tea extract-supplemented food products may be determined by storing samples at normal use conditions and examining them periodically for organoleptic (odor and flavour) changes or by testing them chemically for oxidative rancidity. Although normal use conditions are ideal, this protocol may be too slow to be of practical value. Current methods for determining the effectiveness of green tea extract against lipid oxidation in food are discussed in this section.

8.1. Peroxide value (PV)

The peroxide value (PV), an indicator of primary products of lipid oxidation, has been the most commonly employed chemical assay for evaluating oxidative stability of fats and oils. In this test, the melted fat or oil sample is titrated with a mixture of glacial acetic acid–chloroform (3:2, v/v) and saturated potassium iodide (KI) solution. As hydroperoxides present in the sample oxidize iodide to iodine, the liberated iodine is then titrated with standardized solution of sodium thiosulfate to an end-point. This method is highly empirical, and any discrepancy in the test procedure may result in a variation of results. The PV is calculated by determining all substances which oxidize KI, in terms of milli-equivalents of peroxides per kilogram of sample (Shahidi & Zhong, 2005). These substances are generally assumed to be peroxides or other similar products of oil oxidation. The content of hydroperoxides can be calculated directly by titration of iodine so produced. In order to avoid the use of chloroform, the American Oil Chemists Society (AOCS, 2003) has developed an alternative method, which uses isooctane as solvent (AOCS, 2003; Official Method Cd 8b-90), although the method is limited to PV of less than 70 meq/kg as described in the AOCS guidelines. The results of this test are linked to flavor precursors such as hydroperoxides, but not to the actual flavour compounds. In an internal study, samples of 40% low-fat spreads were produced with green tea extract containing 60 ppm total catechins or 100 ppm TBHQ and stored at 5 °C for 13 weeks. A control sample contained no antioxidant solution. While the hydroperoxides content has significantly increased in the control sample, the samples containing green tea extract or TBHQ had significantly fewer hydroperoxides after 15 weeks of storage. In another study, Mustafa (2013) demonstrated that green tea extract inhibited lipid hydroperoxides formation in ground beef during refrigerated storage (Mustafa, 2013).

8.2. Conjugated dienes

Oxidation of highly unsaturated fatty acids is associated with an increase in the ultra violet (UV) absorption of the product. During lipid oxidation, double bonds in unsaturated fatty acids are changed from non-conjugated to conjugated double bonds. This process takes place almost immediately after hydroperoxides have been formed (Gunstone & Norris, 1983). These conjugated diene hydroperoxides have a UV maximum at 230–240 nm, with a strong characteristic absorption at 232 nm. Formation of conjugated dienes is an indicator of detection of primary changes in lipid oxidation. Compared to peroxide value determination, the measurement of conjugated dienes is much simpler and faster and does not rely on chemical reactions (Shahidi & Zhong, 2005) or formation of color development. The inhibition of formation of conjugated
dienes by green tea extract or other sources of antioxidants can be used as means of assessing antioxidant performance in food. Formation of conjugated dienes is reported to correlate well with peroxide values during the early stages of lipid oxidation (Wanasundara, Shahidi, & Jablonski, 1995). During internal testing on 40% fat spreads, the addition of 300 ppm green tea extract was adequate to inhibit conjugated dienes formation during 13 weeks of storage at 5°C. In another internal study, samples of 40% low-fat spreads were prepared with green tea extracts containing 40 ppm catechins, and 60 ppm catechins or 100 ppm TBHQ solution and stored at 5°C for 15 weeks. The addition of green tea extract had a dose-dependent effect on the inhibition of conjugated dienes. Moreover, the addition of green tea extract with 60 ppm catechins and 100 ppm TBHQ inhibited the development of conjugated dienes most effectively throughout the storage period.

8.3. Thiobarbituric acid (TBA) value

TBA is one of the most widely used methods of assessing the extent of lipid oxidation in foods. The TBA value is typically expressed in milligrams of malondialdehyde (MDA) equivalents per kilogram of sample, as determined by the methods described. This assay is derived from the color reaction between TBA reagent and oxidation products of lipids. Although most food products naturally contain some level of malondialdehyde as a secondary product of lipid oxidation, especially affected foods are fried foods, cheese, cooked meats and dehydrated foods. This assay utilizes the reaction of MDA with TBA to produce a pink MDA–TBA adduct, which has an absorbance maximum at 530–540 (532) nm and molar extinction coefficient of 155 000 M⁻¹ cm⁻¹ (Kinter, 1995). The formation of this adduct requires high temperature (90–100°C) and acidic conditions. The MDA-TBA adduct so formed can be measured spectrophotometrically. The TBA assay has been widely applied in numerous research studies. Close examination of this method, however, began to divulge that a number of compounds other than MDA (i.e., alkenals, alkeniinals, browning reaction products, proteins, sugar degradation products) also react with TBA to form chromogen products, which absorb at approximately 532 nm. Hence, the results of this test can be misleading due to lack of specificity. Thus, the term “thiobarbituric acid reactive substances” (TBARS) is currently used instead of TBA (Shahidi & Zhong, 2005). Nevertheless, this is one of the frequently used protocols to detect oxidative deterioration in lipid-bearing foods. The inhibitory effect of green tea extract on the oxidation of meat lipids was reported by Heš, Korczak, and Gramza (2007), who investigated the effect of this additive on lipid oxidation in minced pork meat under frozen storage conditions. The authors determined the content of TBARS in the control sample and in samples containing 500 ppm tea extract, 500 ppm rosemary extract and 200 ppm BHT. After 6-month storage of samples, the content of TBARS in samples containing rosemary and tea extracts were significantly lower than those found in BHT and control samples. In another experiment with a meat model system, antioxidant activity of green tea catechins as evaluated by TBA values was epigallocatechin gallate > epicatechin gallate > epigallocatechin > epicatechin. The inhibitory effect on TBA values of catechins was concentration dependent, being highest at 200 ppm (Shahidi & Alexander, 1998).

8.4. Oil Stability Index (OSI)

The Oil Stability Index (OSI) is an American Oil Chemists’ Society approved method (AOCS, 1997; Official Method Cd 12b-92) that determines the relative resistance of fats and oils to oxidation. Oxidative stability instrument (Omnion Inc., Rockland, MA, USA) or Rancimat (Brinkmann Instruments, Inc., Westbury, NY, USA) can be employed to determine OSI of oil samples. Both Rancimat and Oxidative Stability Instrument are commercial pieces of equipment for the automated determination of oxidative resistance and are well-known by the fats and oils industry. The same basic principle lies behind both the Rancimat and Oxidative Stability Instrument (Shahidi & Zhong, 2005). The two instruments differ only slightly in their design and operating conditions. In this method, a sample of oil or melted fat is weighed into a glass test tube. The test tube is then placed in a heating block at a temperature of typically around 110°C. However, the OSI may be run at temperatures of 80–140°C. A stream of purified air is passed through the sample, and the effluent stream of air is bubbled through a collection tube containing deionized water. An electrode is placed in deionized water, and the conductivity of the water is continually monitored. As the oil oxidizes, volatile organic acids are produced and trapped in the collection tube, which increase the conductivity of the water. This change in conductivity is monitored by computer software. The Oil Stability Index (OSI) is defined as the point of maximum change of the rate of oxidation. In a study conducted by DuPont Nutrition & Health laboratories, the OSI values of canola oil containing liquid green tea extract were determined at 110°C. The canola oil sample with nothing added had an OSI value of 19.8 h. When liquid green tea extract (1300 ppm) was added to the canola oil, the OSI value increased up to 31.4 h. Hence, canola oil with green tea extract found to be more stable as compared to the control sample with nothing added.

8.5. Oxipres™

The Oxipres™ (Mikrolab Aarhus A/S, Hojbjerg, Denmark), a method of examining accelerated stability of heterogeneous food and feed products, is a modification of the traditional ASTM oxygen bomb method. In this method, the food or feed samples are placed in a closed container with an oxygen headspace. Oxidation is accelerated by heating (to 90–140°C) and by the use of oxygen under pressure (initial oxygen pressure of 5 bars). As the product oxidizes, the oxygen content of the headspace gas decreases which results in a decline of pressure within the closed container. This reduction in pressure is measured electronically. Induction period can be determined by plotting the pressure over time. This induction period (IP) is assumed to be a measure of the resistance of the lipids toward oxidation. For ease of interpretation, the amount of oxygen consumed by the sample can be calculated and plotted against time. Products have a large and fast oxygen consumption (as indicated by short IP) will be more prone to oxidation, and presumably have a shorter shelf-life. The Oxipres™ is normally
equipped with three thermostatic aluminum heating blocks, each with two holes allowing six oxygen bombs to be operated simultaneously at a maximum of three different temperatures. The apparatus may also be equipped with only two oxygen bombs. It is suitable for testing the expected shelf-life of products with multi-phase systems and systems containing water (i.e., margarines, fats, spreads, mayonnaise, salad dressings, snacks, peanuts, fish meal, chicken meal, meat products, etc.), which cannot be tested using the OSI. In a study conducted by DuPont Nutrition & Health laboratories, both control and green tea extract-stabilized fat spreads were made using standard industry practice. Green tea extract was added at a dosage level of 200 ppm. Samples were evaluated for Oxipres™ induction times at 100 °C. Results showed that oxidative stability of fat spreads was significantly improved using the green tea extract. Samples supplemented with green tea extract had higher induction periods as compared to control with nothing added.

8.6. Schaal oven storage test

The Schaal oven test, also referred to as oven storage test, is often used for evaluating oils, fats, and foods containing fats and oils. The AOCS (1997) official method Cg 5-97 describes the protocol for an accelerated test to measure the stability of oil by aging in an oven at 60 °C. Methods such as peroxide value, conjugated dienes, TBARS, sensory evaluation, gas chromatographic volatile compounds are then employed to measure oxidation levels and endpoints in the oils. Because this test uses relatively low temperatures, the samples are exposed to mild oxidative stress. The results of the Schaal oven test normally correlate exceptionally well with actual shelf-life predictions because the end-point represents a lower degree of oxidation (Frankel, 2005). Under Schaal oven test condition at 65 °C over a period of 144-h, seal blubber and menhaden oils treated with tea catechins showed excellent oxidative stability as compared to samples containing BHA, BHT, TBHQ, and α-tocopherol. The antioxidant capacity of catechins in prevention of marine oils oxidation was in the order of epicatechin gallate > epigallocatechin gallate > epigallocatechin > epicatechin. Moreover, the epigallocatechin was slightly more effective than TBHQ in the system used (Wanasundara & Shahidi, 1996).

8.7. Sensory evaluation

Sensory evaluation is a scientific method that is used to measure, analyze, recall, and interpret the reactions to those characteristics of foods as they are perceived through the human senses of sight, smell, taste, touch and hearing. Sensory testing requires panels of human participants, on whom the products are evaluated, and then record the responses made by them. The sensory quality of a food product is one of the most crucial aspects influencing its success in the marketplace. All sensory testing protocols are typically categorized into three broad groups: hedonistic, discriminative and descriptive testing. Among these, descriptive tests are always used within the scope of consumer evaluations and serve to characterize the consumer behavior. On the other hand, both discriminative and descriptive sensory tests may only be carried out by trained/experienced panelists and can give exceptionally detailed information about individual product parameters. Hence, the selection of a proper sensory method is crucial. Sensory evaluation methods are typically expensive and may require a relatively large number of samples. Nonetheless, sensory analyses are typically conducted by the food industry to evaluate the performance of antioxidants during shelf-life of food products. Internal studies conducted by DuPont Nutrition & Health have demonstrated that sensory quality of green tea extract was superior to some commonly used synthetic antioxidants in chilled (4 °C), roasted turkey burgers. The WOF in the control sample within 24 h of storage was unusually high. The WOF of samples treated with green tea extract was not detected even after 13-days of storage at 4 °C. Moreover, the sensory attributes of green tea extract were superior to those of the synthetic antioxidant BHA. In another study, Mildner-Szkudlarz et al. (2009) showed that the sensory profile of green tea extract-supplemented biscuits was superior to samples containing BHA.

9. Conclusions

During the past few decades, the use of natural antioxidants and plant-derived extracts has received increased interest due to concerns over possible adverse health effects caused by the use of synthetic antioxidants. Green tea extract, a natural source of antioxidant, has been successfully used not only to enhance flavour but also to extend the shelf-life of various food products. Green tea extract is particularly suitable for products with high susceptibility to lipid oxidation, including trans-free and low-fat products and foods with a high content of polyunsaturated fatty acids. The primary application areas of green tea extract include meat, poultry, seafood, mayonnaise, salad dressings, soups, sauces, margarines, fat spreads, shortening, frying oils, bakery products, pizza toppings, cereals and snack foods, among many others. Research reports on measuring the effectiveness of green tea extract in food against lipid oxidation are scarce in the literature. The typical parameters measured in determining the effectiveness of green tea extract in food include peroxide values, conjugated dienes, thiobarbituric acid reactive substances (TBARS), Oil Stability Index (OSI), Oxipres induction period, and sensory evaluation, among others.

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