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Phenolic compounds of green tea: Health benefits and technological application in food

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ABSTRACT

Green tea has been an important beverage for humans since ancient times, widely consumed and considered to have health benefits by traditional medicine in Asian countries. Green tea phenolic compounds are predominately composed of catechin derivatives, although other compounds such as flavonols and phenolic acids are also present in lower proportion. The bioactivity exerted by these compounds has been associated with reduced risk of severe illnesses such as cancer, cardiovascular and neurodegenerative diseases. Particularly, epigallocatechin gallate has been implicated in alteration mechanisms with protective effect in these diseases as indicated by several studies about the effect of green tea consumption and mechanistic explanation through *in vitro* and *in vivo* experiments. The biological activity of green tea phenolic compounds also promotes a protective effect by antioxidant mechanisms in biological and food systems, preventing the oxidative damage by acting over either precursors or reactive species. Extraction of phenolic compounds influences the antioxidant activity and promotes adequate separation from green tea leaves to enhance the yield and/or antioxidant activity. Application of green tea phenolic compounds is of great interest because the antioxidant status of the products is enhanced and provides the product with additional antioxidant activity or reduces the undesirable changes of oxidative reactions while processing or storing food. In this scenario, meat and meat products are greatly influenced by oxidative deterioration and microbial spoilage, leading to reduced shelf life. Green tea extracts rich in phenolic compounds have been applied to increase shelf life with comparable effect to synthetic compounds, commonly used by food industry. Green tea has great importance in general health in technological application, however more studies are necessary to elucidate the impact in pathways related to other diseases and food applications.

1. Introduction

Green tea (produced from *Camellia sinensis*) is a popular leaf usually consumed as infusion with pleasant taste with believed positive effect in general health even at high doses of 8–16 cups a day [1]. Leaves of green tea are rich in bioactive compounds,

particularly phenolic compounds with antioxidant activity. The elevated proportion of catechins is related to biological functionality, although recent studies have identified several other phenolic compounds at lower concentration, in particular flavonols and phenolic acids [2,3].

Scientific studies have indicated the effects of green tea consumption in general health and reduction of risk in severe diseases. This is a trend with promising and positive results to assist the control of body weight [4], protection against ultraviolet radiation [5], physical functional performance [6,7], oral health [8], bone health [9] and other physiological effects. Special attention has been given to specific diseases including those with severe effects such as neurodegenerative and cardiovascular diseases. The beneficial effects of green tea

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consumption are associated with polyphenolic compounds that have aroused the interest in food industry and among researchers [10].

The use of phenolic compounds from natural sources in food is an interesting opportunity for the application of biological activities of these compounds, particularly the antioxidant potential, and allows the production of food without synthetic antioxidants for consumers, because the current concern about the impact of food on health has been influencing the consumer choice of food on the basis of its formulation [11]. Synthetic antioxidants are additives commonly used in food industry; however, because of controversial results in literature about the biological effect in some diseases, healthy organizations such as European Food Safety Authority recommended acceptable daily intakes for butylated hydroxyanisole and butylated hydroxytoluene (BHT) of 1.0 and 0.25 mg/kg body weight/day [12,13].

Green tea can be included in the formulation of some products to increase the general antioxidant activity for nutritional or technological purposes. Prevention of lipid oxidation in food can be achieved by several mechanisms in a similar manner as observed in biological structures (*e.g.* free radical scavenging and metal-chelating activity). Lipid oxidation can modify physical–chemical and sensory characteristics such as color, flavor and taste. Among the diversity of food requiring the application of antioxidants, meat and muscle products are particularly affected by lipid oxidation, demanding the addition of antioxidants to extend shelf life [14,15].

This review focuses on the phenolic composition, the antioxidant mechanism by which green tea polyphenols exert antioxidant activity, the biological activity of green tea with potential health benefits, and finally the influence of technology to enhance the extraction of phenolic compounds and the application in food industry.

2. Phenolic composition of green tea

The great interest in green tea composition has been associated with the antioxidant activity and consequently with elevated phenolic content. More recently, a wide diversity of compounds have been identified and several methods were developed to identify and quantify these compounds. Some characteristics of phenolic compounds have been considered for identification of each class of phenolic compounds in several matrices. The thermal sensibility demands techniques such as liquid chromatography instead of gas chromatography, because degradation of important phenolic compounds in green tea can reach 70% at temperatures lower than that usually applied in gas chromatography [16]. The double bonds in the aromatic ring of phenolic allow spectrophotometric measures in UV–visible range. The evaluation of maximum absorption indicates, at least the subclass (*e.g.* flavanol, flavonol and flavones) or supports the identification with a standard. The unique fragmentation pattern of each phenolic compound permits the identification in mass analyzers or a provisional identification for compounds without an available standard, even for complex and high molecular weight compounds [17]. Considering the above characteristics, the liquid chromatography separation followed by spectrophotometry and/or analysis by mass spectrometry can provide valuable information for the investigation of phenolic profile in green tea extracts. Other analyses were also conducted to provide solid information on

the phenolic profile of green tea using nuclear magnetic resonance (NMR) [18].

Flavonoids are a group of phenolic compounds with several sub-classes: anthocyanidins, flavanones, flavanols, flavones, flavonols and isoflavones. These sub-classes have a common basic structure made of 15 carbons with a three carbon bridge connecting two aromatic rings in the configuration C6–C3–C6. Along with flavonoids, phenolic acids are another important group divided in hydroxybenzoic acids and hydroxycinnamic acids. Gallic acid is a relative simple structure also known as 3,4,5-trihydroxybenzoic acid. This compound is the basis of hydroxybenzoic acids and other derivatives with reported antioxidant activity such as ellagic acid. The counterpart, the hydroxycinnamic acid derivatives have the *p*-coumaric acid as basic structure that is formed by an aromatic ring with one hydroxy substitution and one propenoic acid [19].

Studies evaluating the phenolic composition of green tea have provided valuable information about the structure and also about the antioxidant activity (Table 1). The phenolic content is widely diverse, although catechins are the major constituents and other flavonoids and phenolic acids have been identified and quantified.

Table 1

Studies about the identification of phenolic compounds in green tea.

Technique	Number of compounds identified (subclass)	Confirmation with standards
HPLC-DAD-ESI-MS	8 (flavanol)	Yes
LC-MS ⁿ and HPLC-MS-SPE-NMR	9 (flavanol) 22 (flavonol)	No
LC-DAD-ESI-MS	6 (phenolic acid) 6 (flavanol)	Yes
UPLC-DAD-ESI-MS	5 (phenolic acid) 17 (flavanol) 27 (flavonol) 12 (phenolic acid) 4 (other phenolics)	Yes
HPLC-DAD-ESI-MS	5 (flavanol) 8 (flavonol) 4 (phenolic acid)	Yes
UHPLC-MS/MS	8 (flavanol)	Yes
LC-ESI-MS ⁿ	5 (flavanol) 9 (flavonol) 2 (phenolic acid)	Yes
HPLC-DAD	7 (flavanol)	Yes
LC-DAD-MS	4 (flavanol) 5 (flavanol) 2 (phenolic acid) 1 (other phenolics)	No
HPLC-DAD	7 (flavanol) 1 (phenolic acid)	Yes

2.1. Flavanols

The importance of flavanol content in tea phenolic composition leads to quantification of total and individual flavanols that include gallo catechin, catechin gallate, gallo catechin gallate, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate [20,21]. This flavonoid subclass is the most abundant in phenolic composition of green tea, accounting for more than 70% of total phenolic content, as reported in several studies [18,22,23]. The online antioxidant activity of individual phenolic compounds of green tea measured by

Stewart *et al.* [24] also indicated flavanol group was responsible for more than 92% of antioxidant potential. In the recent study conducted by Spáčil *et al.* [25], major catechins were identified by UHPLC-MS/MS with adequate repeatability, reproducibility and sensitivity that can reduce time required and degradation during analysis and enhance the throughput demanded in complex mixtures.

2.2. Flavonols

Flavonol group is another important constituent in green tea [23]. In a recent study, van der Hooft *et al.* [18] evaluated the flavonol content in green tea and reported the presence of several glycoside structures (glucose, galactose, rhamnose, rutin and *p*-coumaric moiety) of kaempferol and quercetin as observed for kaempferol-3-*O*-(glucose-(1,3-rhamnose-1,6-glucose)) and quercetin-3-*O*-(glucose-(1,3-rhamnose-1,6-galactose)). In this study, elucidation of conjugated phenolic compounds was achieved by combination of LC-MSⁿ and HPLC-MS-SPE-NMR, providing valuable information because both the complexity of compounds identified and the scarcity of authentic standards do not allow the confirmation for all compounds identified. In a recent study, several acylated glycosylated flavonols were identified in green tea composition but in low concentration (around 0.36 mg/g) compared with flavanols. These compounds presented kaempferol as the basic structure, with acetyl and *p*-coumaroyl moieties linked to hexosyl and hamnosyl structures forming compounds as kaempferol 3-*O*-*p*-coumaroylglucoside and kaempferol 3-*O*-*p*-coumaroyldirhamnosylhexoside [23].

2.3. Phenolic acids: hydroxybenzoic and hydroxycinnamic acids

This subclass is less expressive for green tea leaves, and the concentration of compounds included in this group is usually lower than that observed for flavanols. The presence of phenolic acids such as gallic acid, *p*-coumaric acid and quinic acid derivatives, caffeoylquinic acid isomers, and caffeoyl glucose is reported in literature, contributing to comprehension of phenolic composition of green tea [18,22,26].

3. Antioxidant mechanism

Antioxidant activity is expressed as the capacity of a molecule or ion to avoid oxidative reactions of other molecules. Phenolic compounds present in green tea leaves exert antioxidant potential by different mechanisms, providing additional protection against oxidants and providing additional protection against oxidative reactions and reactive species. The oxidative series of events proposed by Miguel [27] provides an overview about the major effects of antioxidants (preventive and primary antioxidants), which may also be presented by green tea extracts rich in polyphenols. Preventive antioxidants can exert capacity against oxidative reaction by decreasing the local oxygen concentration, avoiding chain reaction initiation by scavenging radicals (*e.g.*, HO•, O₂•⁻), preventing the generation of radicals and breaking down lipid peroxides to peroxy and alkoxy radicals. Primary antioxidants exert activity in posterior events inducing the decomposition of peroxides to nonradical products and inhibiting hydrogen removal from oxidable by intermediate radicals such as peroxy and alkoxy radicals. These radicals are part of the

reactive oxygen species that are involved in oxidative damage of biological and food systems. The major impacts are related to lipid and protein oxidation, membrane damage, mutagenesis and carcinogenesis, which are of great importance to evaluate how natural extracts impact and reduce these effects [28].

Tests carried out *in vitro* provide interesting results about the mechanism involved in antioxidant activity of green tea phenolic compounds. Several tests can be applied to quantify the antioxidant activity in green tea based on hydrogen atom transference, electron removal and prevention of lipid oxidation. The group of analysis based on hydrogen atom transfer includes tests such as the oxygen radical absorbance capacity (ORAC) assay and total radical-trapping antioxidant parameter assay. The other group of analysis, which involves the electron transfer, consists of Folin-Ciocalteu reagent test, ferric ion reducing antioxidant power, Trolox equivalence antioxidant capacity and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. Additionally, the activity of phenolic compounds has been tested against reactive oxygen species associated with oxidative damage in human body (*e.g.*, peroxy radicals, superoxide anion and hydroxyl radical) [27,29]. An important outcome observed in studies about phenolic compounds in green tea extracts is the correlation between phenolic content and antioxidant activity assessed by multiple methods [30–32]. Structural differences among phenolic compounds in green tea also play important role for antioxidant activity. In the study conducted by Socha *et al.* [33], the individual flavanol content of green tea was inversely associated with radical content of green tea leaves. Epigallocatechin gallate presented higher correlation coefficient than other tested flavanols. This outcome was associated with the presence of hydroxyl group in aromatic rings of galloyl and galloyl substituents because flavanols without this substituent displayed reduced antioxidant activity.

Scavenging of radicals such as hydroxyl and superoxide radicals is an important preventive action associated with polyphenols of green tea, as reported by Guo *et al.* [34]. Individual phenolic components of green tea showed capacity to scavenge hydroxyl radical at different levels. Epicatechin gallate presented higher capacity to scavenge hydroxyl radical than epicatechin, epigallocatechin gallate and epigallocatechin. In a recent study, Kaviarasan *et al.* [35] evaluated the capacity of Sunphenon (a phenolic-rich fraction of green tea) to scavenge free radicals and reported capacity to scavenge singlet oxygen, nitric oxide (NO), O₂•⁻ and hydroxyl radical. However, due to the very low concentration of hydroxyl radical in steady state, this analysis does not possess the practical meaning as expected even with modifications [36], which indicated the use of other radicals [37]. Alternative methodologies to evaluate free radical scavenging activity include relative stable radicals such as DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) radical assay. These two radicals are very popular and applied to several natural extracts and synthetic compounds, including green tea extracts.

Evaluation of green tea phenolics to scavenge DPPH and ABTS⁺ radicals is well characterized in literature. The comparative study of several types of commonly consumed tea performed by Oh *et al.* [38] evaluated green tea extracts by DPPH and ABTS⁺ radical assays. The highest antioxidant activity was observed for ethanolic and aqueous extracts for both radicals. In addition to the elevated radical scavenging activity of major catechin derivatives in green tea, structural differences also influence the radical scavenging activity in DPPH. This outcome was reported by Nanjo *et al.* [39], which

indicated the galloyl substituents present in epigallocatechin gallate and epicatechin gallate were related to higher scavenging activity than epigallocatechin and epicatechin. Similar outcome was also reported by Salah *et al.* [40] for ABTS⁺ radical.

The capacity to scavenge peroxy radical is a measure by ORAC assay. Green tea polyphenols also act as peroxy radical scavengers as indicated by the positive correlation between this assay and total phenolic content. Antioxidant activity of green tea measured by ORAC evaluation suggested high capacity to scavenge peroxy radicals *in vitro* [41,42]. Green tea polyphenols also present quenching activity against singlet oxygen. Mukai *et al.* [43] showed green tea catechin and catechin derivatives could quench singlet oxygen (catechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate) and particularly epigallocatechin gallate and epicatechin gallate presented similar overall rate constants (combination of physical quenching and chemical reaction) observed for α - and γ -tocopherol.

Metal-chelating activity is also reported for green tea. Carloni *et al.* [2] compared the metal-chelating activity of white, green and black tea from the same cultivar. Despite the elevated antioxidant activity and catechin content among tea samples, green tea presented the lowest metal-chelating activity among all samples. This result and the lack of correlation between metal-chelating activity and antioxidant activity suggesting other phenolic compounds in green tea are associated with this capacity. However, in the study conducted by Venditti *et al.* [44], green tea also presented metal-chelating activity lower than white tea and higher than black tea. These authors also indicated the correlation between metal-chelating activity and antioxidant activity.

Phenolic composition also plays a central role in metal-chelating activity. Khokhar and Owusu-Apenten [45] evaluated the effect of structure activity of phenolic compounds and the capacity to chelate iron. These authors indicated the presence of galloyl group does not increase the ability to bind iron in catechin molecule, which means catechin has greater iron-binding activity than epigallocatechin gallate. This effect is also dose dependent for catechin because the increase in proportion of epigallocatechin gallate in reaction promoted a reduction of iron-binding capacity, which explains, in part, the metal-binding activity of phenolic compounds in green tea. The studies about the antioxidant activity of green tea phenolic compounds through different mechanism suggest the potential for technological application, particularly in food, as natural alternatives for synthetic antioxidants.

4. Health benefits

Epidemiologic studies about the benefits of green tea consumption against important diseases, supported by *in vitro* and *in vivo* experiments, reported promising results about the protective effect of green tea. Catechins, as major phenolic constituents in green tea, are also the compounds associated with health benefits by modulation of relevant mechanisms altered by important diseases as reviewed in this section.

Cardiovascular diseases are among the main causes of death, accounting for almost one-third of all deaths worldwide [46]. This alarming situation has led several researchers and physicians to study this disease and search for relevant information to diminish the risk and reduce the number of new cases. In this scenario, green tea can exert an important

preventive effect in cardiovascular system as suggested by epidemiologic studies. Sano *et al.* [47] evaluated the intake of green tea and the incidence of cardiovascular disease and reported that an elevated daily consumption of green tea in patients without cardiovascular disease than those with cardiovascular disease (5.9 and 3.5 cups, respectively). Wang *et al.* [48] related green tea consumption to lower risk of coronary artery disease in Chinese patients. In this study, the risk was inversely associated with green tea consumption and presented a dose dependent effect by increasing regularity, period and intake of green tea.

One of the major effects of green tea consumption is the increase of catechin and catechin derivative levels in human plasma, which seems to be dose dependent and specific for each phenolic compound. Renouf *et al.* [49] evaluated the effect of three different doses of green tea in healthy subjects and observed a dose-response between low and medium-dose levels (180 and 300 mg of total catechin, respectively) of green tea consumption. However, the dose-response was not observed for high dose (415 mg of total catechin content). This interesting effect was related to the saturation of (–)-epigallocatechin and 4'-O-Me-epigallocatechin in plasma. Additionally, (–)-epigallocatechin gallate and (–)-epicatechin plasma levels were dose dependent and presented increasing plasma levels proportional to ingested green tea dose.

Interestingly, the chronic consumption of green tea displays a different behavior as reported by Fung *et al.* [50]. In this experiment, plasma levels of selected catechin derivatives were evaluated after 1 and 2 h of green tea consumption and after 7 days of daily consumption. The plasma level of epigallocatechin gallate after 1 h of tea consumption was the highest among catechin derivatives evaluated, followed by epigallocatechin and epicatechin gallate that remained elevated even after 2 h of green tea consumption. In the chronic consumption evaluation, an unexpected result was observed because only epicatechin gallate presented higher level in plasma.

Once catechin derivatives are present in plasma, these compounds may exert cardioprotective effect reducing the risk of cardiovascular disease by acting over low-density lipoprotein (LDL) [51]. Suzuki-Sugihara *et al.* [52] reported the increased amount of gallate catechins in human plasma after consumption of green tea and its accumulation in LDL fraction of healthy subjects. This accumulation allowed that gallate catechin exerts direct antioxidant activity on LDL, which suggested a protective effect against atherosclerosis. In contrast, Koutelidakis *et al.* [53] evaluated the effect of green tea consumption in patients with coronary artery disease in controlled diet and observed no significant changes in biomarkers during postprandial time. However, total antioxidant capacity increased after 1.5 and 3 h of tea consumption and triglyceride levels decreased after 3 h, which are considered important biomarkers linked to cardiovascular disease. Peroxidation of human LDL can be also prevented by green tea polyphenols. Liu *et al.* [54] showed that epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate were effective in preventing peroxidation of LDL. The authors also supported the following mechanism: initiating and/or propagating peroxy radicals are trapped by phenolic compounds. Additionally, green tea phenolics act on endogenous α -tocopherol by reducing α -tocopheroxyl radical to active antioxidant form. These outcomes suggest catechin, by means of catechin derivatives, as a dietary

source of bioactive compounds associated with protective effects against cardiovascular diseases.

Cancer is a major cause of death worldwide with more than 14.1 million new cases and 8.2 million deaths in 2012 [55]. Most common tissues related to cancer in humans are prostate (in men), breast (in women), lung/bronchus, and colorectal as suggested by large studies based in United States and European countries, although stomach and pancreatic cancers as well as leukemia were also major causes of deaths [56,57]. The elevated number of cases and deaths is linked to risk factors such as smoking [58–60], increased body weight [61,62], inadequate diet [63,64] and lack of physical activity [65,66].

Despite the great impact of cancer in population around the world, green tea consumption has been associated with reduced risk on several types of cancer. In the large-scale study in urban Shanghai population, green tea consumption was associated with reduced pancreatic cancer risk. Increased consumption of tea, regular consumption for long periods, and lower temperature of tea were the main factors related to reduction of pancreatic cancer risk for women and nonsmoking men. Particularly for regular consumption habits, the risk of pancreatic cancer is reduced by 32% in women [67]. The study performed by Hsu *et al.* [68] reported the inverse association of green tea consumption and the risk of nasopharyngeal carcinoma among Taiwan population.

The Shanghai Women's Health Study is an important study about the women's health that evaluated 69 310 women for 11 years to assess the consumption of tea and cancer risk. The results for middle-aged and older Chinese women with regular consumption of green tea presented an inverse association with cancer development for all digestive system. The risk for digestive system cancers combined is reduced by 21% for women with regular consumption of two to three cups/day. Interestingly, cancer risk in digestive system was also reduced once the amount of tea and time of regular consumption were increased [69]. Regarding prostate cancer, Kurahashi *et al.* [70] showed that green tea consumption of five or more cups/day was inversely related to prostate cancer risk in advanced stage compared with men with low green tea consumption (less than one cup/day).

However, this trend for cancer risk reduction is not observed in some studies. Montague *et al.* [71] reported the lack of association between green tea consumption and reduction of prostate cancer risk in 27 293 Chinese men in Singapore. The assessment of tea drinking habits revealed no association between daily consumption and reduction of prostate cancer. Iwasaki *et al.* [72] studied the association of green tea consumption and breast cancer risk in Japanese women and observed no association between green tea drinking habit and reduction of breast cancer.

Following the suggestion of epidemiologic studies about green tea consumption and reduced cancer risk, several studies have investigated which pathways are influenced by green tea phenolic compounds in many types of cancer cells. However, mechanisms by which green tea consumption reduces cancer risk in human tissues remain unclear even with epidemiologic evidences and recent studies showing promising results to elucidate this association.

Cerezo-Guisado *et al.* [73] evaluated the effect of epigallocatechin gallate to induce mitogen-activated protein kinase and Akt pathways of human colon adenocarcinoma cell line HT-29. This flavanol promoted an increase in phosphorylated

forms of ERK1/2, JNK1/2, and p38a, p38g, p38d, and Akt levels and also promoted cell death in HT-29 cell. However, the authors reported a partial association between this pathway and the apoptotic effect of epigallocatechin gallate because in the presence of specific inhibitors for Akt, ERK1/2, and p38, mitogen-activated protein kinase did not inhibit apoptosis of HT-29 cell. Zhang *et al.* [74] evaluated the effect of epigallocatechin gallate in HCCLM6 hepatocellular carcinoma cell and HL-7702 noncancerous liver cell line. In this study, treatment with epigallocatechin gallate reduced the expression of Bcl-2 and nuclear factor- κ B as possible pathways for apoptotic mechanism. Additionally, this study indicated that epigallocatechin gallate increased the expression of Bax (regulates apoptotic signaling), p53 (gene altered in cancer cells), caspase-9 and caspase-3 (control of cell death), and release of cytochrome *c* (protein associated with inner layer of mitochondrial membrane). In a recent study, Thakur *et al.* [75] suggested the cell cycle arrest and apoptotic effects induced by green tea polyphenols involved the suppression of class I histone deacetylases in prostate cancer cell lines (LNCaP cells and PC-3 cells). Green tea promoted the inhibition of class I histone deacetylases and its protein expression along with cell cycle arrest at G₀–G₁ phase and apoptosis in a dose-dependent manner for concentrations between 10 and 80 μ g/mL.

In animal model, cell cycle arrest and apoptosis effects are also observed for metabolites of green tea from live animals. Zhang *et al.* [76] reported induction of apoptosis and cell cycle arrest in rat hepatoma cells (AH109A cell line) and murine melanoma cells (B16 cell line) from green tea phenolic metabolites (epigallocatechin gallate, epigallocatechin and epicatechin gallate) extracted from rat treated with green tea supplementation.

Reduction of insulin-like growth factor-I (IGF-I) activity is a mechanism influenced by green tea polyphenols because this pathway is associated with the inhibition of cancer cell proliferation. Shimizu *et al.* [77] reported the inhibitory effect of epigallocatechin gallate (20 μ g/mL) in IGF-I expression of colon cancer cell (SW837 cell line). Vu *et al.* [78] assessed the effect of epigallocatechin gallate (at 100 μ mol/L) in human pancreatic carcinoma cells and reported a reduction of cell proliferation, which was related to inhibition of IGF-I activation. Adhami *et al.* [79] observed reduction of IGF-I activity in transgenic adenocarcinoma of the mouse prostate model by green tea extract. This study described the effect of continuous administration of green tea polyphenol supplementation during cancer development over 24 weeks, which reduced IGF-I levels and increased IGF-binding protein-3 concentration. The authors also suggest that green tea phenolic compounds use the IGF-I/IGF-binding protein-3 signaling pathway, which is one of the main mechanism to inhibit cancer development and prevent prostate cancer angiogenesis and metastasis.

The risks of neurological diseases are also influenced by green tea consumption. Some studies have suggested a favorable effect of green tea consumption in neurological disorders and reduced cognitive deficits. Although the mechanisms by which green tea polyphenols act in neurological disorders are not yet fully elucidated, promising results are present in literature, particularly for Parkinson's and Alzheimer's diseases. Parkinson's disease is a neurodegenerative disease with major effect on movements and body balance. The major characteristics are rhythmic tremors (resting, postural and kinetic) and oscillatory movement of a body part, although nonmotor symptoms are also observed (*e.g.*,

depression, anxiety and dementia). The occurrence is centered in population older than 40 years and can present prevalence from 100 to 300 cases for 100 000 people [80,81]. Epidemiological studies about Parkinson's disease have suggested an inverse association with tea and green tea consumption. Tanaka *et al.* [82] evaluated the association between Parkinson's disease and consumption of Japanese and Chinese tea (including green tea). Results indicated an inverse association of tea consumption and Parkinson's disease occurrence. Interestingly, this effect also suggested caffeine consumption, in a dose-dependent manner, was inversely related to Parkinson's disease occurrence. However, Tan *et al.* [83] also reported no association of green tea consumption and Parkinson's disease in the Singapore Chinese Health Study and also associated consumption of black tea (with relevant caffeine content) as a possible dietary factor inversely associated with Parkinson's disease. Guo *et al.* [84] reported the protective effect of green tea in rats with induced Parkinson's disease by 6-hydroxydopamine. In this study, green tea extracts protected dopaminergic neurons in a dose-dependent manner (at 150 and 450 mg/kg) against increase of NO and reactive oxygen species, lipid peroxidation, nitrite/nitrate content, inducible NO synthase and protein-bound 3-nitro-tyrosine by exposure to 6-hydroxydopamine. Additionally, radical scavenging activity of midbrain and striatum was preserved by green tea extract. Prevention of inducible NO synthase activity was significantly reduced in mouse model of Parkinson's disease treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. This study also observed a reduction in the number of neuron death (rate of less than 50%) [85].

Alzheimer's disease is an important age-related disease and is estimated to affect the life of 24 million people in elderly population. The symptoms are related to brain functions such as memory and thinking skills that gradually degrade and can evolve to severe cognitive difficulty, compromising the patient's ability to perform simple tasks [86,87]. Consumption of green tea, at high daily dose, was related to lower prevalence of cognitive impairment in 1 003 elderly Japanese (70 years or higher) in the comprehensive geriatric assessment from 2002 [88]. At mechanistic level, green tea activity influences central mechanisms of Alzheimer's disease such as extracellular deposition of amyloid- β peptide and hyperphosphorylation of tau protein causing reduction of brain and cognitive functions. Chan *et al.* [89] observed that rats with accelerated senescence treated with green tea (1% in diet) showed cognitive enhances compared with control group. Increase in serum antioxidant activity, reversion in cognitive impairment, reduction in spongy degeneration and lipofuscin were the main effects of green tea supplementation.

Amyloid- β peptide can form insoluble plaques leading to neuron death and therefore dementia. In this sense, Bastianetto *et al.* [90] reported the effects of green tea extract and catechins on reduction of toxicity induced by amyloid- β peptide in rat hippocampal cells. It was observed that both epigallocatechin gallate and gallic acid inhibited aggregation of amyloid- β and/or formation of neurotoxin ligands derived from this peptide. Posteriorly, Lee *et al.* [91] associated the reduced secretion of amyloid- β peptide to epigallocatechin gallate through modulation of extracellular signal-regulated kinase and nuclear transcription factor- κ B pathways in mice. Additionally, this study highlights positive effects of green tea extract such as reduction of brain degenerative changes and aging process.

Once tau protein is hyperphosphorylated, the activity of this protein is disrupted and the regulation of axonal transport is compromised. These events cause the accumulation of toxic species of soluble tau and neurofibrillary tangles [92]. Inclusion of green tea extract (50 mg/kg) in drinking water of Alzheimer's transgenic mice (Tg2576) reduced the sarkosyl-soluble phosphorylated tau isoforms and improved working memory of treated animals [93]. Lee *et al.* [94] noticed the pretreatment with green tea for 4 weeks in adult Sprague–Dawley rats provided protection against damage of neurons in primary hippocampus by okadaic acid. In this study, hyperphosphorylation of tau protein was reduced, suggesting the protective effect. Wobst *et al.* [95] reported epigallocatechin gallate inhibited the aggregation of stable, toxic and oligomeric tau fragment (K18 Δ K280) *in vitro*. The effect decreased the aggregation of K18 Δ K280 fragment by 10–100 folds. The combination of these effects provides valuable information and elucidates, in part, how green tea may present protective effect against Alzheimer's disease.

5. Technological aspects of green tea in food

Green tea is a good source of antioxidants in diet, although technological application in food has shown promising results in diverse processed food. However, an important factor to consider is the efficiency of phenolic extraction from tea leaves, which can be enhanced by adequate technology. Use of green tea polyphenols as antimicrobial and antioxidative agents in food demands thorough evaluation of characteristics altered in food by this natural extract. Elaboration of clean label food is an advantage from inclusion of natural extracts in processed food, which demands less rigorous safety evaluations than synthetic counterpart [96]. This condition is also a good opportunity for application in meat and meat products that are greatly affected by oxidative reactions.

5.1. Influence of technology applied on extraction of phenolic compounds

The choice of an adequate solvent for phenolic extraction has great impact on separation of phenolic compounds from green tea leaves because the interaction between solvent and analytes has great impact on yield and posterior quantification. Bastos *et al.* [26] tested the efficiency of water, ethanol and ether to extract polyphenolic compounds from green tea leaves and observed higher extraction capacity for ethanol and water than ether, which means ethanol and water have higher interaction with phenolic compounds than ether. Additionally, the radical scavenging activity presented elevated values (around 90% of inhibition of DPPH radical) in all extracts, suggesting the phenolic compounds in green tea extracts presented elevated antioxidant activity even in low concentration. The sequential extraction procedure with methanol and 70% acetone was evaluated by Manian *et al.* [97] in green tea leaves. This combination was effective to extract high amount of phenolic compounds (72.4 and 47.6%, respectively). The 70% acetone solution proved to be very effective and suitable solvent for extraction of phenolic compounds because the lower amount of recovery (around 3%) removed 47.6% of phenolics, even after the first extraction with methanol.

Time of extraction also influences the amount of phenolic extracted as described in the study performed by Rusak *et al.* [30], who indicated at least 15 min of contact between green tea

leaves and solvents. In this period, the extraction with loose green tea leaves of both water and water with lemon juice provided the highest phenolic content (around 2000 mg gallic acid equivalent/L). The highest phenolic content was also observed after 30 min for these two solvents and for 70% ethanol solution. The extraction with bagged green tea leaves also presented highest phenolic content (around 2000 mg gallic acid equivalent/L) after 15 min but using 40% ethanol solution in extraction.

The impact of extraction technique on removal of phenolic compounds presents conflict results in literature. Sultana *et al.* [10] compared the efficiency of traditional extraction (solvent reflux equipment), microwave-assisted extraction and the “Aquisolv” equipment extraction to extract phenolic compounds from green tea leaves from India, Japan, China and Ceylon and commercial product “Teefix” using tap and distilled water. Despite the differences of the three procedures tested, no significant differences were observed, even for tea leaves from different locations and solvents used. Considering the short time required for microwave-assisted extraction, this technique seems to be advantageous. In contrast, Spigno and De Faveri [98] reported increased phenolic content of green tea extract using a household microwave oven. In this study, the microwave power, time of extraction and ratio of solvent/solid were evaluated to optimize extraction process. The yield of phenolic extraction was increased when the time of extraction was superior to 150 s and the potency was higher than 450 W. Efficiency of extraction at highest potency tested (900 W) caused a three-fold increase in a recovery of phenolic compounds compared with conventional brewing process after 210 s of extraction. The ration of solvent/solid was evaluated at two conditions: for constant volume and for constant mass. In the condition of constant volume, the increase of green tea amount caused enhancement of recovery, but for constant mass experiment, the yield of phenolic extraction displayed an increasing behavior between 20 and 50 mL/g and after 50 mL/g, it decreased. Interestingly, this effect was associated with temperature of tea, which presented similar behavior to phenolic recovery. The extraction by a conventional microwave oven did not show deleterious effect on phenolic compounds. The authors also suggested that microwave heating procedure for industrial application demands more studies, but at domestic scale, this technology seems promising.

The ultrasound technique for extraction of green tea phenolic shows advantages due to simplicity of equipment demanded and reduced cost. The response surface study reported by Lee *et al.* [94] showed the optimal condition for extraction of phenolic compounds by use of 19.7% ethanol solution, extraction time of 26 min at temperature of 24 °C. This outcome was indicated by DPPH assay, since the extract obtained in such condition consumed 82% of DPPH radical. This optimized condition seems to be very attractive because low temperature, short time of extraction and low amount of ethanol were required to achieve optimal conditions.

5.2. Application of green tea extracts in meat and meat products

Technological application of green tea extracts in meat and meat products has positive effects on inhibition of both lipid oxidation and microbial spoilage, which are major goals for extension of shelf life. In the study performed by Lorenzo *et al.*

[99], green tea extract inhibited the development of undesirable microorganisms (total viable count, lactic acid bacteria, *Pseudomonas* spp., and psychrotrophic anaerobic bacteria) in porcine patties at 1000 mg/kg after 20 days in modified atmosphere (80% O₂–20% CO₂). In lipid oxidation evaluation, this extract presented similar effect to synthetic antioxidant BHT in thiobarbituric acid index after 12 days of storage at 2 °C, which suggests this green tea extract is natural alternative to synthetic antioxidants. In the comparative study performed by Lin *et al.* [15], pepperoni sausage was produced with BHT (0.02%), green tea extract (from 0.02% to 0.05%), nitrite (between 0.003% and 0.015%) and combination of green tea and nitrite (0.05% and 0.009%, respectively). After 76 days, all individual antioxidant treatments prevented lipid oxidation compared with control, but the highest effects were observed for 0.05% of green tea extract and nitrite at 0.009%, compared with control samples (0.53, 0.42 and 0.79 mg malondialdehyde/kg sample). The combination of green tea extract and nitrite presented pro-oxidant effect because this treatment presented similar values to control samples after 76 days of storage.

Fermented meat products are appreciated worldwide and are produced by growth of specific strains of microorganism in crude meat, particularly lactic acid bacteria, responsible to develop desirable sensory properties and physical-chemical characteristics. Neffe-Skocińska *et al.* [100] reported no significant effect of green tea extract over the development of probiotic *Lactobacillus rhamnosus* LOCK900 counts after 21 days of ripening process, suggesting no interference of green tea extract on microbial growth. The development of expected strains of bacteria in fermented meat products is of great importance because these microorganisms avoid the development of deteriorative and pathogenic microorganisms, increasing the security for fermented meat products. Bozkurt [101] also observed positive effects of green tea application in sucuk (a traditional Turkish dry-fermented sausage). Green tea extract promoted a significant reduction on lipid oxidation of sucuk, compared with samples without antioxidant and presented higher capacity to inhibit lipid oxidation than BHT batch. Additionally, this author observed the reduced content of putrescine (biogenic amine) in sucuk prepared with green tea. Biogenic amines are quality indicators associated with food poisoning and deleterious effects in health if consumed in high concentrations [102].

Protein oxidation is commonly observed during storage in meat and meat products. Although the involvement of phenolic compounds in protein oxidation is not fully elucidated, protein oxidation seems to be inhibited by phenolic compounds [103,104]. The study performed by Jongberg *et al.* [105] showed that the green tea extract reduced lipid oxidation and the level of carbonyl formation in bologna-type sausages elaborated with oxidatively stressed pork, but failed to diminish the thiol loss and protein crosslink (indicators of protein oxidation). The evaluation of sausages by electron spin resonance spectroscopy revealed the presence of radicals, probably originated from protein-bound phenoxyl radicals, exerts a double effect: prevention of carbonyl formation and inducing alterations in protein thiols. In another study, Jongberg *et al.* [14] assessed the effect of different concentrations of green tea extract in a meat emulsion system and observed a limit of 100 mg/L of green tea in this system that inhibited lipid and protein oxidation. Also, textural stability was not compromised by this level of green tea, but at higher concentrations of 500 and 1500 mg/L, texture modifications and oxidative instability were more evident.

Color, particularly red, is perceived as a major influence in expected quality of meat by consumers [106]. In this sense, preservation of characteristic red color is important during storage of meat and meat products. Jo *et al.* [107] assessed the effect of irradiated and nonirradiated freeze-dried green tea powder (0.1%) in raw and cooked pork patties. Lipid oxidation of both raw and cooked patties was inhibited by irradiated and nonirradiated freeze-dried green tea powder. Additionally, the intensity of red color after antioxidant treatments was higher than that of control (without antioxidants) during storage. However, in some studies, the inclusion of green tea extract promoted loss of redness [99,108], which demands more studies to explain this controversial effect.

Technological use of green tea extract is not limited to the protection of meat and meat products as functional ingredient, but can be applied in films and form active packages. This technology has advantages because it does not modify product formulation and allows the use of modified atmosphere. Siripatrawan and Noipha [109] evaluated the effect of chitosan film prepared with green tea extract (20%) in shelf life of commercial pork sausages. Lipid oxidation and microbial growth were reduced in samples wrapped with phenolic enriched film, compared with pork sausage wrapped with common film, prolonging the shelf life of commercial pork sausages. In the experiment conducted by Lorenzo *et al.* [110], positive results for green tea extract in food-grade synthetic film extended the shelf life of foal steaks in modified atmosphere (80% O₂–20% CO₂). Spoilage of microbial growth was reduced during 15 days; however, the effect on lipid oxidation was less intense than expected, which can be attributed to the elevated concentration of oxygen (associated with increased lipid oxidation).

5.3. Application of green tea extract in other food

Traditional infusion of green tea leaves possesses strong antioxidant activity compared with other beverage sources of dietary antioxidant such as some types of wines, beer and chamomile tea [111]. The advances of food industry have provided different beverages and ways of consumption of green tea. Besides the commercial bagged design to be prepared in home with hot water, production of beverages with green tea extract increased consumption of this beverage, providing products rich in phenolic compounds and potential sources of natural antioxidants. However, additional antioxidant ingredients are added to increase the beverage stability [112,113]. The effects of green tea fortification (at 2% and 4%) in the characteristics of commercial yogurt were studied by Jaziri *et al.* [114], who observed no effect on yogurt characteristic microorganism and lactic acid level, which are important parameters during milk fermentation in yogurt production. Additionally, the development of yogurt bacteria did not affect the catechins from green tea. In this case, green tea fortification presented promising results for yogurt manufacture.

However, in some cases, addition of green tea extract causes significant changes in properties of food, as observed by Ahmad *et al.* [115] for cookies elaborated with green tea powder in wheat flour. This powder enhanced the stability and visco-elastic and functional properties of wheat dough, although the water and oil absorption capacity of fortified wheat flour as well as the foam stability were reduced. Sensory analysis revealed increase on acceptability for color, aroma and taste due to green tea powder. The cookies manufactured with green tea powder also presented

higher radical scavenging activity and reducing power than those elaborated with nonfortified wheat flour.

Inclusion of nanocapsules of green tea catechins is a potential application in food. Rashidinejad *et al.* [116] evaluated the encapsulation of green tea polyphenols (catechin and epigallocatechin gallate) and the application in low-fat hard cheese. This study indicated that filling soy lecithin liposomes with green tea polyphenols produced stable nanocapsules. Nanocapsules containing green tea extract were successfully retained in low-fat cheese, which is a new and interesting food matrix for delivering green tea phenolic compounds in diet.

6. Conclusion remarks

The wide and potent phenolic content of green tea has positive impact on health and presents protective effect against severe diseases by multiple mechanisms. More studies are necessary to evaluate the effect in other diseases and mechanisms involved in designing new therapies and the bioactivity of minor polyphenols in human health. In food research perspective, increase of polyphenol extraction and inclusion of green tea polyphenols with high capacity to prevent oxidative reactions and other undesirable changes in food are major goals to enhance intrinsic antioxidant potential. Particularly for meat and meat products, natural antioxidants have positive impact to increase shelf life and lipid stability and also provide food without synthetic antioxidants.

Conflict of interest statement

We declare that we have no conflict of interest.

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