Asian Journal of Chemistry; Vol. 26, No. 8 (2014), 2191-2198

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.16057

REVIEW

Isolation and Bioactivities of Main Functional Components in Tea

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Received: 17 July 2013; *Accepted*: 1 November 2013; *Published online*: 15 April 2014; AJC-14990

The main functional components of tea consist of theanine, γ-amino butyric acid, tea polysaccharides, tea polyphenols and caffeine. These functional components contribute to the health benefits for humans mainly through drinking tea, which include anti-oxidation, anti-virus, cancer prevention, blood pressure and blood fat adjustment, learning and memorizing capacities improvement, immunity enhancement, *etc.*, along with the unique and pleasant aroma and flavor, which make tea the most popular beverage around the world. This paper presents a review on the isolation and bioactivities of these functional components naturally existing in tea.

Keywords: Tea, Theanine, γ-amino butyric acid, Tea polysaccharides, Tea polyphenols, Caffeine.

INTRODUCTION

Tea is one of the oldest and most popular drinks consumed by over two-third of the world's population. Traditionally, tea was drunk to improve blood flow, eliminate toxins, and to improve resistance to diseases[1-4]. The tea industry in China also occupies an important place and plays a useful part in the national economy. Tea derives from ancient China and is also the most popular drink in modern China. Although customs of tea drinking in different areas of China are usually different. Generally speaking, people in south China like drinking green tea, oolong tea and black tea, while people in north China like drinking jasmine tea and green tea.

No matter what kinds of tea, they all contain functional components whose health benefits have been proven by scientific experiments and practice of normal life. It is those functional components that make tea not only the most popular beverage in the world but also a natural antioxidative agent available in everyday life, which may help to prevent a wide variety of diseases such as cancers and heart diseases[5-8]. The prominent antioxidative activities of tea derived from the combination of the antioxidative activities of several kinds of functional components.

These functional components in teas are composed of theanine, γ-amino butyric acid, tea polysaccharides, tea polyphenols and caffeine, *etc.* Every kind of these functional components usually has not only common bioactivities such as anti-oxidation activity, but also its unique bioactivities. With revealing the new values for health promotion of these components, the demand for tea in market has been increasing gradually and steadily.

With the development of chemical industries, several kinds of those functional components such as theanine *etc.* can be produced through chemosynthesis. Chemosynthesis has its advantages of low cost and convenient separation *etc.* However, considering the main food applications of those functional components and the easy availability of tea, the most safest and convenient method of obtaining tea functional components is to extract them from naturally planted tea. As a result, more and more attentions focused on the extraction and purification of those components from tea. At the meantime, the values on health promotion of those components have been proven and new bioactivities and values of them have also been revealed.

Theanine

Extraction of theanine: Tea leaves usually contain 1 to 2 % of theanine (dry weight), which is one of the unique non-protein amino acids existing mainly in tea. Many methods have been developed to extract and purify theanine from tea and many relevant papers have been published, although these approaches still have limitations and were not perfect.

Conventionally, chemical agent precipitation method was used to isolate theanine. This approach was complicated and the product was inclined to be polluted by Pb^{2+}, Cu^{2+} and S^{2-} ions. Thus, new approaches including chromatogram methods were developed to extract and isolate theanine.

Zhang *et al.*[9] used preparative HPLC method to isolate and purify theanine from green tea. The concrete procedures were as follows: after the extraction of catechin with ethyl acetate (EA), the green tea residual mass, was dissolved using 50 °C hot water and purified with a 732 cation resin column to get green tea extracts containing 50 % theanine. Then through preparative HPLC, the extracts were isolated and purified to theanine product with higher than 98 % purity. The preparative separation of theanine was conducted by injecting 1.5 mL of filtered 50 % theanine extract solution onto a Water Prep Nova-pak HR C18 reversed-phase HPLC column (19 × 300 mm, 60 Å, 6 μm) using a formic acid mobile phase buffered to pH 3.0. The yield of theanine in the preparative HPLC approach reached 70.4 %.

Lachová *et al.*[10] utilized molecularly imprinted polymer (MIP) technology to selectively purify theanine from green tea extracts and the technique was summarized as follows[11]: two separate MIP formulations were prepared from Nylon-6 dissolved in formic acid using phase inversion techniques. The polymers were then washed with acetic acid solution to remove the template from the generated imprint cavities. The selectivity of theanine rebinding to the MIP was then evaluated by solid phase extraction.

Preparative HPLC method and MIP technique of the purification of theanine might be suitable for the extraction of theanine for chemical analysis, but not suitable for the isolation of theanine on large scale.

Furthermore, a number of patents have also been developed and published covering theanine extraction and purification[12,13].

Bioactivities of theanine: As a unique amino acid existing only in tea and in very few other plants, theanine not only contributes to a pleasant flavor in tea but also provides significant health and cognitive benefits. In addition, theanine was absorbed quickly in the intestinal tract and showed physiological activities[13] and no studies associated with theanine toxicity in human or animal models have been found and reported[11].

Mental relaxation: Findings from several studies have revealed that theanine consumption has been closely associated with mental relaxation.

Theanine has been reported to promote the generation of α-waves on the brain, providing the body a sense of relaxation without inducing drowsiness. α-waves are known to indicate an awake, alert and relaxed physical and mental condition[14,15]. In addition, theanine is reported to promote the release of the inhibitory neurotransmitter γ-amino butyric acid (GABA), which in turn regulates dopamine and serotonin levels in the brain[16].

Kimura *et al.*[17] reported that theanine intake resulted in a reduction in the heart rate (HR) and salivary immunoglobulin A (s-IgA) responses to an acute stress task relative to the placebo control condition. Moreover, analyses of heart rate variability indicated that the reductions in heart rate and s-IgA were likely attributable to an attenuation of sympathetic nervous activation, suggesting that the oral intake of theanine could cause anti-stress effects *via* the inhibition of cortical neuron excitation.

Learning ability enhancement: It has been shown that administration of theanine has a significant effect on the release or reduction of neuro-transmitters like dopamine and serotonin. It is also known that these neurotransmitters are closely related to memory and learning ability. Therefore, the effect of theanine on memory and learning ability was investigated and the animals' results revealed the positive effects of theanine on memory and learning ability[14]. Other findings have also revealed that theanine consumption has been closely associated with improved learning ability[18-21].

In addition, studies have shown that consumption of theanine in combination with caffeine could further improve concentration and learning ability. For example, the intake of a combination of 250 mg L-theanine and 150 mg caffeine was found to enhance rapid simple reaction time, fast numeric working memory reaction time and improve verification accuracy during reading tasks[19].

Another study found that consuming a combination of 100 mg L-theanine and 50 mg caffeine improved both speed and accuracy performance during attention-switching tasks performed 60 min after ingestion and reduced susceptibility to distracting information in memory tasks at 60 and 90 min following ingestion[20].

Cancer prevention: Theanine has also recently proved to be linked to cancer prevention[22,23]. In addition, theanine has been reported to enhance the anti-tumor efficacy of chemotherapeutic agents[24]. Theanine significantly enhanced the inhibitory effect of the cancer treatment drug, doxorubicin (DOX) on tumor growth and increased the DOX concentration in the tumor, compared to DOX-alone group. Moreover, the combination of theanine with DOX suppressed the hepatic metastasis of ovarian sarcoma. Furthermore, theanine can also reduce the adverse effects of the cancer treatment drug, doxorubicin by providing protection against damage caused by doxorubicin to normal tissue[25].

Immune system improvement: Recent studies have also found that theanine was associated with enhancement of the immune system[26].

Cell-mediated and humoral immune responses are attenuated with aging. Intracellular glutathione (GSH) levels also decrease with aging. Takagi *et al.*[26] have reported that combined administration of L-cystine and L-theanine enhances antigen-specific IgG production, partly through augmentation of GSH levels and T helper 2-mediated responses in 12-week-old mice. These findings suggest that combined administration of L-cystine and L-theanine to aged mice improves immune responses *via* increase of GSH synthesis and enhances immune function, and may become a useful strategy in healthy aging.

Blood pressure adjustment: It is well known that the regulation of blood pressure is highly dependent on catecholaminergic and serotonergic neurons in both the brain and the peripheral nervous systems[27]. It was demonstrated that theanine reduced serotonin levels.

Juneja *et al.*[14] thought theanine might have a calming effect on the mental state by lowering blood pressure. Other researchers' findings also revealed the effect of theanine on adjustment of blood pressure[28,29].

The effect of theanine on the blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) was investigated by intraperitoneally administering theanine[28]. When spontaneously hypertensive rats were injected with various amounts of theanine (0, 500, 1000, 1500, and 2000 mg/kg), the change was dose-dependent, and a significant decrease in blood pressure was observed with the high doses (1500 and 2000 mg/kg). The brain 5-hydroxyindole level was also significantly decreased by theanine administration to both Wistar kyoto rates and spontaneously hypertensive rats, with the decrease being dose-dependent.

Extraction of γ-Amino butyric acid: Extraction of γ-amino butyric acid (GABA) is a non-protein amino acid, existing in normal kinds of teas, even though the amount of γ-amino butyric acid is usually very few and the contents of it vary in different kinds of teas.

Zhao *et al*.[30] investigated the comparison of γ-amino butyric acid contents in various types of tea. The results of their investigation revealed that pu-erh tea has a lower γ-amino butyric acid content than green, black, oolong, or white tea. In addition, they also found that the γ-amino butyric acid content in white tea significantly higher than that in the other types of teas. But several previous studies have reported that pu-erh tea contains a high level of γ-amino butyric acid[31,32].

However, in γ-amino butyric acid tea, the content of γ-amino butyric acid can be as high as several hundreds mg per 100 grams, that is over two hundreds times higher than that of normal tea. Japanese researchers first developed γ-amino butyric acid tea which contained affluent γ-amino butyric acid. The γ-amino butyric acid tea, also called Gabaron tea, is the special tea enriched with γ-amino butyric acid by anaerobic conditions of fresh tea leaves[33,34]. Usually it could be called γ-amino butyric acid tea when the content of γ-amino butyric acid in tea exceeds 1.5 mg/g.

There is little information available regarding the optimal extraction conditions of γ-amino butyric acid tea on bioactive components including γ-amino butyric acid and antioxidant properties[35], because γ-amino butyric acid tea was usually directly and conveniently consumed through drinking by tea drinkers, rather than extracting γ-amino butyric acid existing in γ-amino butyric acid tea. In addition, it is not economical to extract and purify γ-amino butyric acid alone from γ-amino butyric acid tea. Lin *et al*.[35] reported the effects of various concentrations of ethanol and extraction temperatures on the extraction yields, total phenols, various catechins, γ-amino butyric acid and theanine of γ-amino butyric acid tea leaves. They found that water at 50-75 °C gave higher γ-amino butyric acid and theanine contents and higher chelating ability of extracts and the optimal extraction conditions to maintain the total contents of various catechins, γ-amino butyric acid and theanine in the maximum level were 50 % ethanol (v/v) and 75-95 °C, based on dried tea extracts or leaves results obtained.

Huang *et al*.[36] reported the extraction and purification of γ-amino butyric acid from γ-amino butyric acid tea using 732 cation-exchange resin. The results indicated that water extraction was better than alcohol extraction with 20 % higher γ-amino butyric acid content. The maximum static adsorption quantity of 732 cation-exchange resin to γ-amino butyric acid was 32.9 mg/g. The adsorptive quantity achieved 70 % within the first 1 h. The pH and flow rate of sample liquid could influence the adsorption quantity; the optimal conditions were 3 (pH) and 3 mL min^{-1} (flow rate). As high as 94.68 % elution rate of γ-amino butyric acid could be obtained when citric acid buffer and $NH_3{\cdot}H_2O$ were used for eluting and the buffer pH was 8.0-9.0.

Bioactivities of γ-amino butyric acid: γ-Amino butyric acid has a physiological role in many systems outside the central system, such as regulation cardiovascular functions, inhibition metastasis of cancer cells, and modulation renal function[35,37].

Mental relaxation: γ-Amino butyric acid could work effectively as a natural relaxant and its effects could be seen within 1 h of its administration to induce relaxation and diminish anxiety[38].

The effect of orally administrated γ-amino butyric acid on relaxation and immunity during stress has been investigated in humans[38]. γ-amino butyric acid significantly increases α-waves and decreases β-waves compared to water or L-theanine. These findings denote that γ-amino butyric acid not only induces relaxation but also reduces anxiety.

Blood pressure adjustment: γ-Amino butyric acid is known to be involved in the regulation of blood pressure by modulating the neurotransmitter release in the central and peripheral sympathetic nervous systems[39,40].

Abe *et al*.[40] indicated that γ-amino butyric acid tea seemed not only to decrease the established high blood pressure, but also to prevent the development of hypertension in rats which were fed a high salt diet. They reported that γ-amino butyric acid is one of the major inhibitory neurotransmitters in the sympathetic nervous system and to play an important role in cardiovascular function. They also found that with an average γ-amino butyric acid intake of 4 mg/rat per day, blood pressure of rat was significantly decreased after four weeks.

Immunity enhancement: Abdou *et al*.[38] investigated the role of relaxant and anxiolytic effects of γ-amino butyric acid intake on immunity in stressed volunteers. Eight acrophobic subjects were divided into 2 groups (placebo and γ-amino butyric acid). All subjects were crossing a suspended bridge as a stressful stimulus. Immunoglobulin A (IgA) levels in their saliva were monitored during bridge crossing. Placebo group showed marked decrease of their IgA levels, while γ-amino butyric acid group showed significantly higher levels. In conclusion, γ-amino butyric acid could work effectively as a natural relaxant and its effects could be seen within 1 h of its administration to induce relaxation and diminish anxiety. Moreover, γ-amino butyric acid administration could enhance immunity under stress conditions.

Anti-fatigue effect: Kanehira *et al*.[41] reported that consummation of γ-amino butyric acid-containing beverages, especially those containing 50 mg of γ-amino butyric acid, may help to reduce both psychological and physical fatigue and improve task-solving ability.

Furthermore, findings from many studies have revealed that γ-amino butyric acid was linked with the decrease of blood pressure, improvement of the function of brain, improvement of the functions of liver and kidney and enhancement of long-term memory.

Extraction of tea polysaccharides: Extraction of tea polysaccharides mainly exist as a structural constituent of cell wall of tea, so the extraction technique depends on the cell wall structure. In general, tea polysaccharides were extracted through solvent extraction method, using water, acidic solution and diluted alkali solution after pretreatments as solvent agents.

Wang *et al.*[42] obtained crude tea polysaccharides from low grade green tea through hot water extraction. The concrete steps could be summarized as follows: 250 g of the lower quality green tea was mixed with 2000 mL of 80 % ethanol (v/v) and heated under refluxing condition at 30 °C for 24 h. Such treatment was repeated twice. After filtration, the tea leaf residues were extracted with 2000 mL distilled water in bath at 90 °C for 2 h. After filtration, the residues were extracted again with 2500 ml distilled water at the same temperature for another 2 h. Then the extracts were centrifuged to remove the contaminants. The supernatant was concentrated through rotary evaporation and precipitated using 95 % ethanol. Then the precipitation was dissolved with purified water and dialyzed in order to remove the small molecules. The dialyzed solution was then freeze-dried and polysaccharide powder was obtained.

In order to obtain purified tea polysaccharide, other equipments and procedures should be essential including chromatography. Nie and Xie[43] summarized the procedures for separating and purifying tea polysaccharides as follows: by extracting with hot water from tea after removing the fat and small molecules by treating by 80 % ethanol, the solution of tea polysaccharides was obtained after filtering and concentrating. By removing the free protein using the Sevage method with chloroform and 1-butanol, and then precipitating with ethanol, filtering, and drying, the crude tea polysaccharides were obtained. After dissolving the crude extracts and decolorizing, the resultant solution was applied to different column chromatography, such as ion-exchange chromatography, gel filtration chromatography or affinity chromatography, eluting with an appropriate running buffer, concentrating, dialyzing and freeze drying, the pure tea polysaccharides can finally be obtained.

Bioactivities of tea polysaccharides: Tea polysaccharides were found to be mostly glycoconjugates in which a protein carries one or more carbohydrate chains covalently attached to a polypeptide backbone, usually *via* N- or O-linkages. In recent studies, the water-soluble tea polysaccharides are demonstrated to have many bioactivities, such as, reducing blood pressure, blood sugar and blood fat levels, immunity adjustment, anti-radiation, anti-blood coagulation, anti-cancer, anti-HIV and hypoglycemic activities[43].

Anti-oxidation: Chen *et al.*[44] reported the components and antioxidant activity of polysaccharide conjugate (TPC) from green tea, which has been used to cure diabetes in China. The results indicated that polysaccharide conjugate exerted significant inhibitory effects on hydroxyl and superoxide radicals and lipid peroxidation with IC_{50} values of 101, 145 and 238 μg mL^{-1}, respectively. Polysaccharide conjugate could also enhance the activity of superoxide dismutase (SOD).

Chen *et al.*[45] utilized absorbent chromatography and ion exchange chromatography to isolate and purify three fractions of water-soluble polysaccharide conjugates, named as TPC-1, TPC-2 and TPC-3 from low grade green tea. TPC-3 showed the highest antioxidant activity among the three fractions of polysaccharide conjugates. They also suggested that the molecular weight and protein content of the polysaccharide conjugates improved the bioactivities significantly.

Wang *et al.*[42] investigated the anti-oxidant activities of tea polysaccharide from leaves, flowers and seeds of green tea (TLPS, TFPS and TSPS respectively) and the results revealed that TLPS, TFPS and TSPS exhibited strong anti-oxidation in a concentration-dependent manner. Furthermore, TLPS and TFPS had higher antioxidant activity than TSPS. They suggested that the characteristics of monosaccharide and the higher molecular weight in TLPS and TFPS molecules may contribute to the activities.

Lowering blood sugar, blood lipid and blood pressure: The crude tea polysaccharides (CTP) and a tea polysaccharide fraction (TPF) were found that could significantly decrease fasting blood glucose (FBG) and glucosylated serum protein (GSP) in alloxan-induced diabetic mice[46].

Other investigations demonstrated that the tea polysaccharides possess the strong hypolipidemic effects[47,48]. In addition, findings also revealed that tea polysaccharides could lower the blood pressure and heart rate, and increase coronary blood flow rate of rats.

Anticoagulant and antithrombotic effects: Wang and Wang[49] reported that the tea polysaccharide could significantly prolong the clotting time both *in vivo* and *in vitro*. The results showed that tea polysaccharide had a high blood anticoagulation activity, significantly prolonged the time to thrombosis, and shortened the length of thrombosis, which was involved in the anticoagulant and antithrombotic effects.

Anti-cancer: The role of tea in protection against cancer has been supported by many evidences from the studies in cell culture and animal models. And emerging animal and clinical studies are beginning to suggest that the tea polysaccharides may play an important role in the prevention of cancer.

The anticarcinogenic effect of tea polysaccharide was investigated by using a panel of short term cell biology assays[50], the cytokinesis block micronuclei test in V79 cells induced by mitomycin, the test of metabolic cooperation between V79 and M cells and the test of growth ability of Hela cells in soft agar were used in the screening. The results showed that the tea polysaccharide tested were effective in the test involved in different stages of carcinogenesis, *i.e.* initiation, promotion and progression.

Nie *et al.*[51] investigated the antioxidative and anticancer activities of tea glycoprotein (TGP). The results revealed that dose-dependently tea glycoprotein exhibited good antiproliferation activity to one kind of colon cancer cell lines (HCT-15), whereas exhibited very weak antiproliferation activity to another one (Caco-2).

Radiation protection: Wan[52] stated that in 1970s, Tea Research Institute of Chinese Academy of Agricultural Sciences and Center for Disease Control and Prevention of Tianjin reported the radiation protection effects of tea polysaccharide products with acute radiation sickness prevention experiments. According to this experiment, after subcutaneous administration of tea polysaccharides, the mice were irradiated by ^{60}Co at the irradiation dose of 766-840-lun (R). The results showed that the tea polysaccharide had significant protection ability against radiation, with 30 % increase for the survival rate of mice.

Extraction of tea polyphenols: Green tea leaves contain about 10-30 % (w/w) tea polyphenols. Tea polyphenols include catechines, flavanols, flavanones, phenolic acids, glycosides and the aglycons of plant pigments, which are soluble in water, ethanol, methanol and acetone *etc*.

The conventional methods of the extraction of tea polyphenols included solvent extraction and ionic precipitation approaches.

Ionic precipitation was based on the fact that metal ions and tea polyphenols could produce precipitation. Firstly, tea was leached with hot water or ethanol and then solutions after filtration was adjusted pH to be alkaline. Secondly, precipitants [such as Cu(OH)Ac, $Ca(OH)_2$] were added to solutions after pH adjustment and then precipitation produced. Solutions after filtration could be used to extract caffeine and precipitations were dissolved with H_2SO_4 solution and extracted using ethyl acetate. Organic phase was selected and crude tea polyphenols could be obtained through vacuum drying.

The concrete processes of solvent extraction usually indicated as follows: tea was leached using solvent (hot water or ethanol), solutions after filtration was concentrated and extracted using chloroform. Then water phase was kept and extracted with ethyl acetate. Organic phase was then collected and through vacuum drying crude tea polyphenols could be obtained. The conventional solvent extraction method could be summarized as Fig.1.

Based on basic extraction methods, new techniques and equipments were also utilized to extract and purify tea polyphenols.

Sakanaka[53] reported using lignocellulose to extract caffeine-free tea polyphenols. A process for isolating tea polyphenols and caffeine from green tea leaves using water extraction, followed by ultrafiltration with a CA-Ti composite ultrafiltration membrane, adsorption by PA resin and finally elution by a mixed solvent system has been developed[54].

Pan *et al.*[55] utilized a microwave-assisted extraction (MAE) method for the extraction of tea polyphenols and tea caffeine from green tea leaves. Microwave-assisted extraction can greatly reduce the extraction time for the same extraction. From the points of extraction time, the extraction efficiency and the percentages of tea polyphenols or tea caffeine in extracts, MAE was more effective than the conventional extraction methods. Li and Jiang[56] also developed a microwave-assisted extraction of green tea polyphenols. Microwave-assisted extraction of tea phenols from green tea was investigated through orthogonal array design.

Chang *et al.*[57] reported a novel custom-designed supercritical fluid extraction technique coupled with an absorption system to extract polyphenols from green tea leaf.

Bioactivities of tea polyphenols: According to the previous studies, four main tea polyphenols, epigallocatechin gallate (EGCg), epicatechin gallate (ECg), epigallocatechin

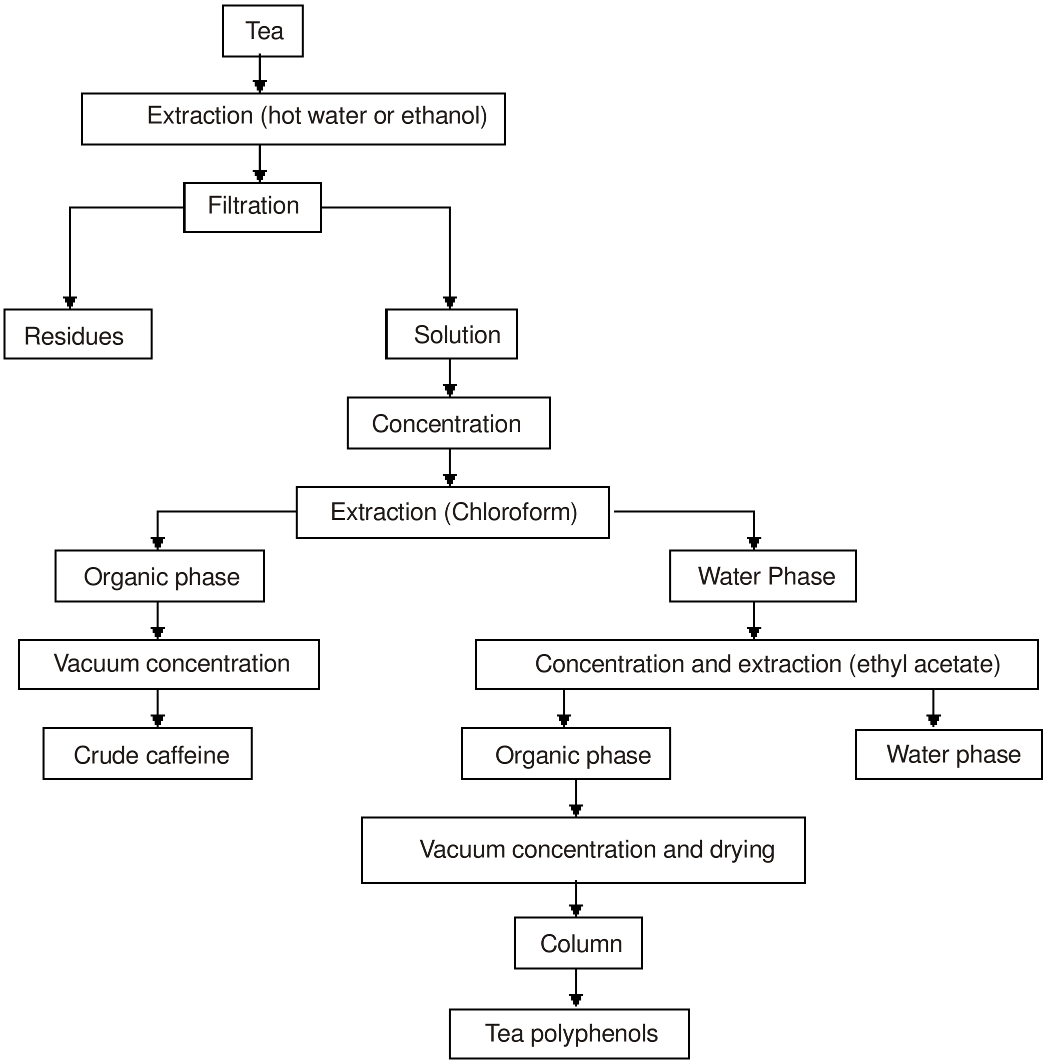

Fig. 1. The extraction process of tea polyphenols and caffeine

(EGC), and epicatechin (EC) are significant antioxidant constituents.

Anti-oxidation activity: Tea polyphenols isolated from green tea leaves, are natural antioxidant[58-59] and have a scavenging effect on active oxygen radical[60].

Similarly, tea polyphenols in other kinds of tea are also natural antioxidants which have prominent antioxidant activities[61,62].

Anti-cancer: Findings of several studies revealed that high doses of theaflavin, black tea or green tea polyphenols, or EGCg reduce tumor multiplicity and green tea infusion also inhibits forestomach chemically induced cancer in mice[63-65]. Dreosti *et al*.[66] reported that heterocyclic amine induced aberrant crupt formation in rat colon can be inhibited by green tea, EGCg, or a black tea polyphenol fraction.

Hibasami *et al*.[67] reported that Oolong tea polyphenol extract could induce poptosis in human stomach cancer cells. Kuroda and Hara[68] also reported the antimutagenic and anticarcinogenic activities of tea polyphenols. Landis-Piwowar *et al*.[69] reported a novel prodrug of the green tea polyphenol (-)- Epigallocatechin-3-Gallate as a potential anticancer agent.

Extraction of caffeine: In general, a tea leaf contains 2-4.5 % caffeine, which is one of the three main methylxanthines (caffeine, theophylline and theobromine) in tea. Actually, the conventional and normal extraction process was figured out in Fig. 1, and the extraction process in Fig. 1 belonged to liquid-liquid extraction. The extraction of caffeine from aqueous solution was generally done using chloroform or methylene chloride, solvents known as possible human carcinogens.

Murray and Hansen[70] developed a less toxic alternative for treatment of tea leaves through the use of water/1-propanol/sodium chloride ternary system, being a suitable replacement for the more traditional water/organochlorine solvent systems.

Saldaña, Mazzafera, and Mohamed[71] have extensively studied the extraction of caffeine from mate tea leaves using methylene chloride, water and carbon dioxide. However, the use of chemical solvents involves the risk of leaving toxic residues in the extracted products while the use of water results in a nonselective extraction and the loss of valuable flavor components. Carbon dioxide, which has a low critical temperature (304 K) and is nontoxic, has become a universally attractive alternative solvent in the extrac-tion of natural products.

Recently, supercritical techniques under pressure that combine solvent extraction and stripping operations in a single process are gaining considerable attention for separating and purifying caffeine from tea leaves[72].

Içen and Gürü[73] investigated the effect of ethanol content on supercritical carbon dioxide extraction of caffeine from tea stalk and fiber wastes. They reported that parameters of ethanol flow rate, extraction time, extraction temperature, carbon dioxide flow rate, process pressure and particle size affected caffeine leaching from tea wastes. The maximum yield of caffeine from tea stalk wastes and fiber wastes were 14.95 mg/g tea stalk and 18.92 mg/g tea fiber, respectively. The almost the same yield reached within 2 h instead of 7 h when the supercritical extraction conditions used of ethanol as cosolvent have been compared with the conditions used only carbon dioxide. Besides saving time and the amount of carbon dioxide, the supercritical extraction yield increase using cosolvent had been recorded as 62.5 and 63.1 %, respectively, in comparison with the conventional extraction method of tea stalk and fiber wastes using chloroform.

Xi[74] developed a new method using high pressure processing to extract caffeine from green tea leaves. The effects of different parameters such as high hydrostatic pressure (100-600 MPa), different solvents (acetone, methanol, ethanol and water), ethanol concentration (0-100 % mL/mL), pressure holding time (1-10 min) and liquid/solid ratio (10:1 to 25:1 mL/g) were investigated for the optimal caffeine extraction conditions from green tea leaves. The highest yields (4.0 ± 0.22 %.) were obtained at 50 % (mL/mL) ethanol concentration, liquid/solid ratio of 20:1 (mL/g) and 500 MPa pressure applied for 1 min. Experiments using conventional extraction methods (extraction at room temperature, ultrasonic extraction and heat reflux extraction) were also conducted, which showed that extraction using high pressure processing had several advantages, such as higher yields, shorter extraction times and lower energy consumption.

Besides liquid-liquid extraction, there were several reports on solid-liquid and solid extraction of caffeine.

Senol and Aydin[72] investigated solid-liquid extraction of caffeine from tea waste containing 2 % (w/w) caffeine using water and chloroform as effective solvents. The extraction has been performed at isothermal conditions of 370 K for water and 293 K for chloroform in the battery type extraction plant including both three and five extractors connected in series. An obvious difference in extraction behavior has been observed for two solvents, distinguishing the divergent interactions attributed to the different mechanism of solvent-caffeine aggregation. The high degree of separation of caffeine by chloroform is feasible with the efficiency ranging about two times larger than water in terms of the solvent flow rates tested. The optimum process parameters were estimated through the graphical interpretation of dependently changing variables, composition, flow rate and extraction degree.

Theodoridis and Manesiotis[75] developed the method of caffeine extraction using molecularly imprinted polymer (MIP). This method could be applied to extracting caffeine not only from beverage (including tea) but also from human plasmid. This method could be utilized in the extraction of caffeine with very little content. A molecularly imprinted polymer was prepared with caffeine as the template molecule. Thermal polymerization (60 °C) was optimized, varying ratios of monomer, cross linker and template. The polymer was used as a solid-phase extraction (SPE) sorbent, for selective trapping and pre-concentration of caffeine. Caffeine was loaded on the MIP-SPE cartridge using different loading conditions (solvents, pH value). Washing and elution of the caffeine bound to the molecularly imprinted polymer was studied utilizing different protocols. The extraction protocol was successfully applied to the direct extraction of caffeine from beverages and spiked human plasma.

Bioactivities of caffeine

Inspiration of nervous system and enhancement of learning and memorizing capacities: Caffeine can stimulate human's nervous system. It is well known that the intake of caffeine can enhance the learning and memorizing capacities of animals and humans since many years ago[76]. Simth[77] also summarized the bioactivities of caffeine on human's nerve system.

Caffeine led to faster digit vigilance reaction time, improved Rapid Visual Information Processing (RVIP) accuracy and attenuated increases in self-reported 'mental fatigue'[78].

Anti-obesity: Huang *et al*.[79] found that caffeine can inspire the heat-producing process, inhibit appetite and decrease the deposit of fats. As a result caffeine has the bioactivities of anti-obesity and has the potential utilization in curing obesity.

Anti-cancer: The anti-cancer capacities of caffeine have been acknowledged, although the mechanism still remains not very clear[80].

Negative effects of extra-consuming of caffeine: Much more consumption of caffeine could lead to hypertension[81]. Furthermore, caffeine could have negative effects on bone tissue[82].

Prospect

Extraction of functional components from tea: Till now, extraction of tea functional components usually involves in the utilization of chemical agents, some of which are noxious and can lead to safety problems. More attentions will focus on the extraction methods and techniques using less chemical agents and avoiding very noxious agents. In addition, with existing techniques and methods, only one or two kinds of tea functional components can usually be obtained using only one method or technique. The new approaches and techniques should be investigated and developed in order to be able to get several components during one extraction process. Furthermore, Extraction methods will tend to be more convenient, quick, nontoxic and environment-friendly.

Bioactivities of the functional components in teas: Few researches focused on the poly-functions of those functional components existing in teas, considering the reciprocity of these functional components. However, it is of great importance since those functional components, not just only one component, existed in teas altogether.

With revealing new and more activities of functional components in teas, it can be imagined that more wide applications of those functional components in food industries and other industries could be developed.

ACKNOWLEDGEMENTS

This work was supported by grants from the Introduced Talents Research Program of Shanghai Institute of Technology (No. YJ2013-21).

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