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REVIEW

Efficient extraction strategies of tea (*Camellia sinensis*) biomolecules

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Abstract Tea is a popular daily beverage worldwide. Modulation and modifications of its basic components like catechins, alkaloids, proteins and carbohydrate during fermentation or extraction process changes organoleptic, gustatory and medicinal properties of tea. Through these processes increase or decrease in yield of desired components are evident. Considering the varied impacts of parameters in tea production, storage and processes that affect the yield, extraction of tea biomolecules at optimized condition is thought to be challenging. Implementation of technological advancements in green chemistry approaches can minimize the deviation retaining maximum qualitative properties in environment friendly way. Existed extraction processes with optimization parameters of tea have been discussed in this paper including its prospects and limitations. This exhaustive review of various extraction parameters, decaffeination process of tea and large scale cost effective isolation of tea components with aid of modern technology can assist people to choose extraction condition of tea according to necessity.

Keywords Tea · Biomolecules · Extraction · Optimization

Introduction

Tea is the mostly used daily beverage throughout the world, with estimated daily consumption of more than 3 billion cups (Chen and Zhou 2005). It is valued due to potential health benefits confirmed with preclinical and epidemiological studies, its aroma content, and cultural association. There is increasing demand of tea extract and isolated tea biomolecules in pharmaceutical and food industries as natural antioxidant and for other uses. Variation of processing technique produces varied tea that are of many types: Green tea, Black tea, White tea, Yellow tea, Dark tea, Pu'erh tea and Oolong tea. Mostly each component of any variety of tea is known for some amount of bioactivity or sensory attributes. Tea biomolecules mainly consists of non protein amino acid theanine, free sugars (Unachukwu et al. 2010), methylxanthine or purine alkaloid like caffeine, theobromine, theophylline and theacrine, phenolic acids like gallic acid, and eight other catechins; (+)-catechin (C), (−)-epicatechin (EC), (−)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-catechin gallate (CG), (−)-gallocatechin gallate (GCG), (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG) (Peng et al. 2008). (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epigallocatechin- 3-gallate (EGCG) are the main catechin of green tea. Cumulatively they are often called as polyphenols. Though EGCG is the major tea catechin with most anticarcinogenic property, EGC has highest antioxidant efficacy followed by EGCG, EC, and ECG respectively (Lu and Chen 2008).Alkaloid molecules are responsible for stimulant activity of tea (Horžić et al. 2012). Catechin and caffeine content and sensory attributes determines the quality of tea (Choung and Lee 2011). Nowadays decaffeinated tea is more preferred because caffeine causes irritation in gastrointestinal tract, sleeplessness, cerebral cortex stimulation and excites central nervous system in people (Ye et al. 2007). Still awareness of the antioxidant properties and other health benefit like anti carcinogenic and chemopreventive effect (Fujiki 2005; Chen and Dou 2008; George et al. 2008), anti-hyperglycemic effect (Gornes et al. 1995), anti-obesity effects (Lin and Lin-Shiau 2006), anti diabetic (Sabu et al. 2002), anti ulcer effects (Maity et al. 1995), in cardiovascular diseases (Deka and Vita 2011), anti-arthritis effects (Katiyar and Raman 2011), and anxiolytic effect (Vignes et al. 2006) made this beverage popular

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worldwide. These attributes designates tea polyphenols as functional food too (Liang et al. 2007a). The components responsible for sensory attributes are z-3-hexanol, benzyl alcohol, linalool, 2-pheylethanol, methyl salicylate, geraniol, nerolidol (Xia et al. 2006).

Commercially available tea is mainly hybrid of two types: *Camellia sinensis* var. assamica and *Camellia sinensis* var. sinensis of family Theaceae. The green tea is not allowed to ferment. Steaming and panning are the processes for impeding catechin oxidation by polyphenol oxidase in it used in Japan and China respectively. Black tea requires complete fermentation of leaves while oolong tea is partially fermented during processing. Thearubigins, red–brown colored components, and Theaflavins, a reddish coloured pigment are main constituent of it (Anesini et al. 2008). Some black tea variety includes Broken Pekoe, Broken Orange Pekoe, Flowery Orange Pekoe, Orange Pekoe, Assam, Darjeeling, Nilgiri, Souchong, Lapsang Souchong, Earl Grey, and Keemun while green tea varieties are Jasmine tea, Gunpowder, Formosa Oolong.

Utilization of tea biomolecules in food industry

Nowadays tea polyphenols are considered as one of the most important food additives. It is used in food with higher water content owing to water solubility, to retard oxidation of food component for long time storage as natural antioxidant, in oil-in-water emulsions at pH 5.5. It also possesses better property than l-ascorbate to retain colour of β – carotene. It is used in chewing gums for halitosis and preventing dental caries. L-theanine, having umami taste is used for taste masking in foods (Dufresne and Farnworth 2001). Tea antioxidants can be also used for long term storage of model emulsion (Penman and Gordon 1997).

Heterocyclic aromatic amines (HA) are considered as food generated carcinogen, obtained from meat and produced during meat cooking. It was found that green tea marinade not only reduce HA content by its free radical scavenging property, but also modifies the taste of pan-fried meat and can be used as non alcoholic marinade too (Quelhas et al. 2010). Even green tea extract treatment can extend shelf life of fresh mutton for few days at near 25 ° C temperature inhibiting lipolytic and proteolytic degradation responsible for spoilage (Kumudavally et al. 2008). Tea polyphenols incorporation in foods like biscuit, noodles for value addition as well as stability enhancement are some new trends in food industry (Sharma and Zhou 2011; Li et al. 2012). Incorporation of green tea extract in chitosan film used for active packaging of food product is also new concept in food industry due to its higher mechanical, antioxidant and water vapour barrier properties (Siripatrawan and Harte 2010). Research finding suggests its potential use to prevent spoilage of pork sausages by increasing shelf life may be due to antimicrobial and antioxidant properties of tea extract (Siripatrawan and Noipha 2012). These are some of the recent important application of green tea as antioxidant additive in food industry.

Extraction of bioactive components of tea

Method of bioactive component extraction from tea

Extraction of catechin and caffeine is the major step required for human consumption of tea as beverage. The conventional process of tea brewing to microwave assisted extraction (MAE) of tea polyphenol, are different types of tea extraction techniques only. Many research works are being conducted to maximize the extraction of each bioactive component retaining its best sensory properties as beverage and increasing shelf life of the active components. Tea components extremely vary in each category (Friedman et al. 2005). It varies per geographical distribution, climatic condition, cultivation processes (Peng et al. 2008;, Lee et al. 2010) and age of tea leaves (Jun et al. 2010). Chemical changes occur during food processing, like conversion of tea catechin to theaflavin during black tea fermentation. Standardization as well as optimization of the extraction procedure for all types of tea mentioned is very difficult in this context. It is also raises several research questions regarding stability of those molecules (Ananingsih et al. 2013). It is proved that higher temperature (100 °C) and prolonged extraction time (2 hour) leads to degradation of bioactive molecules of tea (Perva-Uzunalic et al. 2006) due to partial epimerization of epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) into gallocatechin gallate (GCG) and catechin gallate (CG), respectively (Liang et al. 2007a). Therefore, it is necessary to optimize the extraction at as low temperature as possible.

Numerous studies are being performed to increase extraction rate, decrease extraction time and cost, as well as to maximize processing throughput. At increased temperature, and inside body fluid, these antioxidant components which are often used as nutraceuticals or food grade antioxidant are, easily degraded. Kinetics studies are also thus performed to understand the production and degradation of these ingredients at varied parameters. Storage condition like temperature, humidity and type of food affect stability of tea catechins (Ananingsih et al. 2013). Recent technologies of encapsulation have shown promises to increase shelf life of these tea antioxidant molecules for both food and pharmaceutical purposes.

Different conditions like extraction parameters and set-up, variety of tea used, and storage conditions influence

efficiency of an extraction process. Emergence of novel techniques eases the extraction process, minimize extraction cost, time and maximize the yield. Simultaneously, it is tried to retain its maximum antioxidant potential and sensory attributes namely colour, aroma, sweetness reducing proteins and pectin contents.

The major extraction processes used for tea extraction are conventional solvent extraction, ultrasound assisted extraction (UAE), MAE, high hydrostatic pressure (HHPE), supercritical fluid extraction (SFE). (Xia et al. 2006; Nkhili et al. 2009; Friedman et al. 2005; Jun 2009; Jun et al. 2010; Chang et al. 2000; Park et al. 2007; Ding et al. 2006; Jun et al. 2009; Xu et al. 2012). Since the time of Shen Nung, the great emperor who was supposed to initiate tea drinking custom, conventional approach of hot water assisted brewing became main polyphenol extraction technique. In 2000, soxhletion or conventional solvent extraction with 95 % ethanol was considered the best way for total polyphenolic extraction (Chang et al. 2000). With the advent of MAE and UAE, the concept changed. UAE is suggested for better sensory attributes as it can be carried out at low temperature avoiding volatile component evaporation and thermo sensitive degradation of active biomolecules (Xia et al. 2006; Su et al. 2006) while MAE indicates toward better extractability of polyphenol at reduced time and energy consumption (Wang et al. 2010). Though a study showed that tea component does not vary during extraction between 3 to 20 min (Friedman et al. 2005) other studies mostly opposes the idea considering other extraction parameters. The brief of optimization parameters for tea polyphenol extraction have been tabulated in Table 1. Utilization of each types of extraction process will be explored eventually in the next few sections of the paper.

Improvement of sensory qualities of tea as beverage like enhanced aroma production, reduced tea cream formations are part of its extraction enhancement research. Few strategies which can be implemented for quality up gradation has been tabulated in Table 2.

Conventional solvent extraction technique for tea

Conventional system for tea extraction is an old, time and solvent consuming, laborious process with very low yield associated efficiency. The widely used conventional systems for phytochemical recovery are mainly soxhlet, maceration, reflux, and hydrodistillation. Better yield was found when extracted from fresh leaves than the mature one. Refluxing showed better result than maceration (John et al. 2006). Extraction efficacies of this system mostly depend on solvent selection according to polarity, and proper heat treatment. In these cases there remains a chance of thermal degradation of thermosensitive active molecules as well as incomplete extraction. Japanese tea ceremony is an appropriate example of incomplete single stage water assisted extraction, so that same tea is used for repeated extraction of tea catechins. Again large amount of substrate, sorbent and solvent requirement not only increase the production cost, poses a threat to environment during disposal. These limitations have driven people to search for new alternatives like environment friendly green technologies, which reduce chances of environmental hazards.

Ultrasound assisted extraction (UAE) of tea

The mechanism behind UAE is mainly propagation of ultrasound pressure waves within medium followed by cavitation bubble formation. As the bubbles cannot expand after a certain limit, it implodes and micro turbulence is formed, which in turn disrupt cell membrane, enhance permeability of biomass and accelerate solvent dissolution of target component (Shirsath et al. 2012). Ultrasounds probes were found to be more efficient than ultrasonic bath (Horžić et al. 2012). UAE is a preferred mode of tea catechins extraction due to increased efficacy of extraction process at lower temperature retaining their medicinal values. High temperature extraction often leads to degradation of polyphenols and increase protein and pectin extraction which interfere with the organoleptic quality of tea by cream formation. It was found that UAE can increase tea polyphenol yield at 65 ° C, compared with 85 ° C (Xia et al. 2006). These properties often impel us to ignore the limitation of high time consumption and less extraction efficacy.

Microwave assisted extraction (MAE) of tea

An electromagnetic radiation of wavelength 0.001 to 1 m is considered as microwave. Absorption of the transmitted microwave energy by a medium converts it to thermal energy by ionic conduction and dipole rotation. When microwave passes through a biomass, drastic rise in temperature causes vaporization of internal water and in turn disrupt the cell wall and plasma membrane of the biomass. Increased permeability of solvent to cell matrix enhance the yield by dissolution of target molecule at elevated temperature. Optimization of parameters which affect yield of bioactive molecule by MAE are power and frequency of microwave, duration and number of microwave radiation, moisture content and particle size of plant samples, type and concentration of solvent, solid liquid ratio, extraction temperature and pressure (Zhang et al. 2011). The rate of MAE is affected by the above mentioned parameters in following way; microwave irradiation time > microwave intensity > tea/water ratio > number of times for irradiation (Li and Jiang 2010). But as previously mentioned that tea polyphenol also vary on

Table 1 Optimized polyphenol extraction strategies

Tea biomolecule	Extraction condition	Reference
Polyphenols content-in-extracted solids (PES) in black tea	50:1 water-to-tea ratio and 40 min	Chandini et al. 2011
Catechins in Green Tea Leaves	2 h room temperature extraction using a 2 % phosphoric acid-40 % etoh solution	Choung and Lee 2011
Selective extraction and preconcentration of epicatechin	Solid-phase extraction (SPE) by molecularly imprinted polymer prepared from acrylamide monomer	Ding et al. 2006
Catechin extraction from milk tea beverage (Brewed green tea combined with skim milk)	Use of pepsin (40.0 mg/ml)	Ferruzzi and Green 2006
Polyphenol from yellow tea	Extraction with ultrasound probe than ultrasonic bath in 75 % aqueous ethanol medium	Horžić et al. 2012
Total polyphenol (TP)	50 % (ml/ml) of ethanol concentration, 20:1 (ml/g) of liquid/solid ratio and 500mpa of high hydrostatic pressure for 1 min by high hydrostatic pressure extraction (HHPE)	Jun et al. 2009
Major catechins from green tea	50 % (v/v) ethanolic solvent extraction, solvent to tea ratio 20 ml:1 g, 400mpa pressure for 15 min by ultrahigh pressure extraction	Jun et al. 2010
Two scale extraction for production of catechin- enriched tea drinks	Brewing green tea at 1:50 tea/water ratio in water thermostated at 30 °C for 30 min followed by water extraction of leaves by squeezing and then second brewing step in water maintained at 75 °C for 40 min The first brewing step at 50 °C for 10 min. Followed by water extraction of leaves by squeezing and then soaked for 10 min, for the second brewing step, at 80 °C water. The Two-step extraction procedure was repeated three times	Labbé et al. 2008; Bazinet et al. 2007
Extraction of catechin from Longjing tea	400 W, 1 min irradiation duration, 1:15 gm/ml tea to water ratio by Microwave assisted extraction	Li et al. 2010
Total catechins	50 % (v/v) ethanol for 10 min for dry tea, and 75 % (v/v) ethanol for 10 min for fresh tea leaf. For commercial purpose water mediated extraction at 80 °C	Liang et al. 2007
Tea polyphenols	Membrane separation by nanofiltration with G-membrane in 80 % of ethanol solution	Nwuha 2000
Tea catechin	4 min microwave-assisted time, 50 % (v/v) ethanol concentration in water, liquid/solid ratio of 20:1 (ml/g) and pre leaching time of 90 min by microwave assisted extraction	Pan et al. 2003
Aqueous extaction of catechins	Either high temperature (95 °C) and short extraction time (5–10 min), or lower temperature (60 or 80 °C) and longer extraction time (20 min) to avoid catechin degradation and ratio of solvent to material (100 ml:1 g) or lower ratios (40 ml:1 g or 9 ml:1 g) and a multi-step extraction procedure to obtain more catechin	Perva-Uzunalic et al. 2006
Polyphenol extraction from white and black tea	Accelerated phenolic extraction from green tea by lemon juice addition in water extract, for prolonged extraction of catechin with aqueous ethanol (40 %)	Rusak et al. 2008

Table 1 (continued)

Tea biomolecule	Extraction condition	Reference
Epicatechin (EC), epicatechin Gallate (ecg), epigallocatechin (EGC), and epigallocatechin gallate(egcg), the major four catechin from Hawaii tea leaves	Increase in content of four catechins with increasing leaf age, moving from the bud to first and second leaves	Song et al. 2012
Concentration of tea polyphenols by molecular distillation and drying by spray drying	70 °C distillation temperature, 10 ml/min flux, and 1,200 n/min rotational speed for concentration and 170 °C distillation temperature, 3 ml/min feed flux, 30 % feed concentration, and 30 m^3/h wind capacity for drying	Tang et al. 2011
Catechin by water extraction	80 °C for 30 min, a tea particle size of 1 mm, a brewing solution ph <6 and a tea to- water ratio at 50:1 (ml/g) for maximal extraction and tea to- water ratio at 20:1 (ml/g) for cost efficiency along with maximal yield	Vuong et al. 2011b
Total polyphenol	60 % ethanol concentration, solid-to-liquid ratio 1 : 12 g/ml at 80 °C, 10 min under the microwave power of 600 W.	Wang et al. 2010
Catechins From green tea	Ethanol concentration, 50 %; pressure, 490 mpa; and liquid/solid ratio, 20 ml/g by ultrahigh-pressure extraction	Xu et al. 2012
Polyphenols From Iranian tea	20–40 min and 80 °C for EGC, EC and 80 min and 90 °C for catechin, EGC and EGCG	Ziaedini et al. 2010
EC, ECG, EGC, and EGCG	7 min of steeping at a constant 100 °C at pH 3	Zimmermann and Gleichenhagen 2011

different parameters, a large number of studies have been performed on MAE of different varieties and types of tea leaves and still being continued. MAE at 200–230 °C for 2 min, not only extracts 60–70 % of the polyphenols but 40–50 % of polysaccharides and Cutin, a biopolyester also (Tsubaki et al. 2008). The major limitation here is the high temperature which often causes degradation of tea polyphenols.

High pressure processing (HPP) of tea

HPP is quite new technology used in food technology being low temperature technique having microorganism inactivation, as well as freshness retention property. The concept of adiabatic heating has been applied in this type of extraction. The pressure is responsible for increasing permeability and solubility of the extracts. The major disadvantage associated with this process are possibility of structural changes of few food components, and protein degradation (Jun 2009) though this process is often found to increases the shelf life of food products. Another study does not support the concept of structural changes and they recommend the process of ultra-high pressure extraction (UPE) for avoiding the possibility of structural damage of bioactive molecules. Other attributes of UPE suggested by the study are short extraction time with high reproducibility, high extraction efficacy with less impurity, and lowered consumption of energy and solvent with mild extraction condition (Xu et al. 2012). For extraction of tea components, many types of HPP are used. Some of them are ultrahigh pressure extraction (UPE) (Jun et al. 2010) or high hydrostatic pressure extraction (HHPE) (Jun et al. 2009) where pressure ranges from 100 to 600 MPa. When antioxidant activity of tea is concerned, UPE assisted extract have shown the maximum result among other extraction methods (Jun et al. 2011).

Supercritical fluid extraction (SFE) from tea

In fact SFE requires few newer environment friendly green technologies particularly for tea catechin extraction. Higher selectivity, lesser time consumption and avoidance of toxic organic solvents are the key issues related to this processes (Herrero et al. 2006). Above a critical temperature and pressure a fluid is converted into supercritical fluid which possesses lower viscosity and higher diffusivity than liquid. In industrial purpose CO_2 is mostly used. It is abundant in nature, has lower critical point, can be recycled and use of co solvent as well as modifiers enhance extraction yield (Herrero et al. 2006). Tea components are extracted from a packed bed adsorption column with help of fluidized CO_2 by high pressure. Rigorous control of extraction parameters augments simultaneous separation of caffeine from other tea components by this method (Chang et al. 2000). SFE was proved to be the best method for decaffeination process, but limitation

Table 2 Improvement of sensory properties and quality of tea

Processing techniques	Extraction condition	Reference
Role of different type of water on tea quality	deionized water for greatest yield rate and polyphenols with low caffeine, distilled water for increasing the contents of non-ester catechins, AC enhanced the concentrations of ester catechins	Danrong et al. 2009
Processing of cold brew tea with for ease of polyphenols and free amino acid extraction in low temperature water	moisture content of pre dried tea leaves, 50 %; freezing pretreatment, −18 °C three times, each time for 24 h; puffing temperature, 105 °C	He et al. 2011
Enzymatic modification with tannase for better antioxidant and metal ion chelation property as well as sensory property	At concentration of 200 ppm	Lu and Chen 2008, 2009
Improvement of sensory quality like color, brightness, strength and flavor and EGC and EC contect during extraction of CTC and Kangra orthodox tea	tannase mediated biotransformation by *Penicillium charlesii*	Raghuwanshi et al. 2013
To maximize sensory quality of aroma and sweetness attributes of water extract of oolong tea	3-min soaking at 95 °C	Su et al. 2006
Inhibition of pectin and protein extraction and improved extraction of aroma components and glycosidic aroma precursors	Ultrasonic assisted extraction at ultrasonic input power 250 W, extraction temperature 60 °C and extraction time 40 min	Xia et al. 2006

of this method is unavoidable loss of tea catechins (Park et al. 2007). Still number of patents proves efficiency of this method for decaffeination as the process is user friendly, saves energy consumption and caffeine can be easily separated without processes like heating or evaporation (Kim et al. 2007, 2008). When caffeine extraction was tried from tea waste like tea stalk and fiber, this process showed a novel path (Icen and Guru 2009). Even it was shown that recovery of caffeine content from tea stalk was increased when ethanol was used as co solvent, saving both time and amount of CO_2 (Icen and Guru 2010). Supercritical fluid treatment for decaffeination was also shown to improve water brewing of green tea (Hung et al. 2012). The recent research trend of using SFE in tea extraction is to study the possibility of simultaneous decaffeination and chlorophyll extraction from tea (Park et al. 2012).

Subcritical water extraction (SWE) for tea extraction

SWE is also one of the newer methods for tea extraction in toxic organic solvent free approach. It is the method where hot water assisted extraction is performed at 100 ° C to 374 ° C, and at 10 to 60 bar pressure until water is in liquid state. The theory behind the process is at 250 ° C and appropriate high pressure, the dielectric constant of water eventually decreases and becomes similar to that of ethanol which helps in better extraction yield of polyphenol at organic solvent free environment (Herrero et al. 2006). It was found that SWE has better decaffeination efficiency than conventional water extraction approach (Shalmashi et al. 2007).

Decaffeination or caffeine extraction from tea

Decaffeination or specific degradation of caffeine from tea is necessary to cope up with withdrawal effect of caffeine. Either ethyl acetate or methylene chloride assisted solvent extraction or SFE are the commercial methods used for decaffeination of black tea in multi-stage counter-current extraction. Utilization of toxic organic solvents like chloroform, ethyl hexanoate and propyl acetate or selective extraction of caffeine from water extract by organochlorine solvent are also well known processes for decaffeination of tea, but it has limitations like toxic contamination and disposal of solvent (Senol and Aydin 2006). Other environment friendly processes include SFE or resin, and charcoal absorption has the limitation of high initial setup cost (Vuong et al. 2012). Even decaffeination processes utilizing hot water or co solvent assisted SFE is not a good alternative due to loss of valuable tea catechins (Park et al. 2007; Liang et al. 2007). Whenever caffeine extraction is concerned, tea stalk, fiber wastes or

Table 3 Decaffeination during extraction or caffeine extraction

Process utilized	Parameters	Reference
Extraction of Caffeine from Green Tea Leaves	2 h room temperature extraction using a 2 % phosphoric acid-40 % etoh solution	Choung and Lee 2011
Caffeine from tea stalk and fiber wastes from Turkish tea	Mixing speed of 200 rpm, a water/tea wastes ratio of 18.5 (w/w), a temperature of 98.5 °C, a leaching duration of 16 min and a particle size range of 0.250–0.355 mm.	Gürü and Icen 2004
Decaffeination by Polar Cosolvent-Modified Supercritical CO_2 extraction from tea retaining catechins	3,600 g of carbon dioxide at 333 K and 300 Bar for 4 g of tea soaked with 1 g of water	Huang et al. 2007
Supercritical carbon dioxide extraction of caffeine from tea stalk and fiber wastes	Extraction time of 7 h, temperature of 60 °C, CO_2 flow rate of 11 g/min, pressure of 250 bar and mean particle size of 0.202 mm	Icen and Guru 2009
Supercritical carbon dioxide extraction of caffeine from tea stalk and fiber wastes with ethanol	Flow ratio of 5.23 g ethanol/100 g CO_2 (10 g CO_2/min), extraction time of 3 h, temperature of 65 °C, pressure of 250 bar and mean particle size of 0.202 mm.	Icen and Guru 2009
Decaffienation by hot water	1:20 (w/v) solid: liquid ratio at 100 °C for 3 min. Not suitable for black tea due to loss of Catechin content	Liang et al. 2007b
Microwave-enhanced vacuum ice water extraction (MVIE) for decaffeination	Solvent (ml) to solid (g) ratio 10:1, microwave extraction time 6 min, microwave power 350 w, time 2.5 h, vacuum ice water extraction with 87.6 % yield of caffeine	Lou et al. 2012
Microwave assisted extraction of caffeine (4 %)	4 min microwave-assisted time, 50 % (v/v) ethanol concentration In water, liquid/solid Ratio of 20:1 (ml/g) and pre leaching time of 90 min	Pan et al. 2003
Co solvent assisted super critical fluid extraction for decaffeination	Supercritical fluid CO_2 modified with 95 % (v/v) ethanol at 7.0 g per 100 g of CO_2 at 300 bar and 70 °C for 120 min, with unavoidable considerable amount of EGCG loss	Park et al. 2007
Simultaneous decaffeination and chlorophyll extraction using supercritical carbon dioxide (SC-CO_2)	3.0 g of 95 % (v/v) ethanol cosolvent per 100 g of CO_2, 23 mpa pressure, 63 °C temperature and an extraction duration of 120 min for 10 g of green tea leaves	Park et al. 2012
Decaffeination of Koren Tea	Extracting the green tea with water at 80 °C for 40 min, the extract was partitioned with water/chloroform, and then partition with water/ethyl acetate for catechin recovery	Row and Jin 2006
Subcritical water assisted extraction of caffeine of Iranian Black tea leaf	175 °C, water flow rate of 2 g/min, mean particle size Of 0.5 mm at 3 h of extraction	Shalmashi et al. 2007
Caffeine in Hawaii tea leaves	Decrease in caffeine with increasing leaf age, moving from the bud to first and second leaves	Song et al. 2012
Ultrasonic enhanced supercritical fluid extraction for decaffeination	Extraction pressure of 30 mpa, temperature at 55 °C, time of 4 h, 30 % moisture content, and ultrasound power of 100 W.	Tang et al. 2010
Decaffeination from Iranian tea	20–40 min and 80 °C for caffeine	Ziaedini et al. 2010

underutilized matured tea leaves are good alternative (Gürü and Icen 2004; Icen and Guru 2010; Vuong et al. 2011a) but decaffeination process is a challenging job, where retention of catechin activity is needed. A newer approach for degradation of caffeine to non-toxic product was found to be microbial and enzymatic treatment by *Pseudomonas* and *Aspergillus* species (Gummadi et al. 2009, Gokulakrishnan et al. 2007). Adsorption assisted

Table 4 Theanine extraction from tea leaves

Source of Theanine	Extraction parameters	Reference
Theanine by hot water extraction from Turkish tea	tea particle size 150–300 mm; extraction time 25 min and extraction temperature 80 °C	Sari and Velioglu 2011
L-theanine in Hawaii tea leaves	Decrease in L-theanine content with increasing leaf age, moving from the bud to first and second leaves	Song et al. 2012
water extraction of L-theanine,a non-protein amino acid	80 °C, 30 min, water:tea ratio of 20:1 mL/g and a tea particle size of 0.5–1 mm	Vuong et al. 2011a, 2011b

Table 5 Use of adsorption process in different tea biomolecule separation

Process	Utility	Reference
Silica adsorbent containing β-cyclodextrin with methanol/acetonitrile/acetic acid mobile phase system	90 % EGCG recovery	Lai et al. 2012
Ultrafiltration with cellulose Acetate–titanium composite ultrafiltration membrane, adsorption by pa resin and finally elution by a mixed solvent system after water extraction	90 % of tea polyphenol recovery	Li et al. 2005
Chloromethyl, Amino, and Phenylamino Groups Functionalized Macroporous Adsorption Resins	Optimal temperature 338.15 K for maximized tea Catechin extraction and minimized caffeine retention	Liu et al. 2012
Poly(acrylamide-co-ethylene glycol dimethylacrylate) as adsorbent	Favoured adsorption of catechins with 192.85–171.11 mg/gm adsorption capacity and simultaneous decaffeination	Lu 2010
Adsorption by sawdust lignocellulose column	Separation of egcg and Caffeine in acidic conditions (ph 5.9–2.0)	Sakanaka 2003
Methacrylic acid in molecular imprinted polymers as the sorbent materials in solid phase extraction	Caffeine-theophylline Mixture and pentoxifylline-theophylline separation	Wang et al. 2004
Instant tea treatment by activated carbon (AC)	Partial decaffeination	Ye et al. 2009
Catechin adsorption by Woody tea stalk, Pine sawdust and Sugarcane bagasse	Selective tea Catechin adsorption with 209.41, 120.5 and 118.6 mg/gm adsorption capacity respectively	Ye et al. 2009
Macroporous crosslinked Poly(n-vinyl-2-pyrrolidinone) adsorbent with mobile phase ethanol	98 % tea polyphenols and 2 % caffeine recovery by adsorption with 98 mg/gm adsprption capacity	Zhao et al. 2008
Polyvinylpolypyrrolidone as adsorbent, water elution for caffeine and dimethylsulfoxide/ethanol elution for catechins	Separation of Catechin and caffeine	Dong et al. 2011

decaffeination will be thoroughly discussed in adsorption based tea component separation. Various optimization processes of decaffeination or caffeine extraction have been tabulated in Table 3.

Extraction of tea proteins

Protein present in tea causes cream formation and reduces the sensory attributes of tea at higher temperature. So UAE is often suggested for enhancement of sensory qualities of tea, despite having limitations of more time consumption and less extraction efficiency (Xia et al. 2006). In dry base, tea leaves contain 21–28 % proteins, which has biological cell protection activity against irradiation mediated mutagenesis. Glycine, valine, lysine, leucine, alanine, methonine, and threonine contents of tea protein are even higher than soy proteins in terms of g/100 g protein. Alkaline and enzymatic treatment of fresh and polyphenol extracted tea leaves yields a considerable amount of protein, though protein content was lower in exhausted leaves, may be due to degradation or possibility of being washed out during water soaking. Tea proteins can be used in food industry as foaming agent, emulsifying agent, a gelling agent, and as texture modifier (Shen et al. 2008). Theanine is important for a typical sodium glutamate like umami taste, and for reduction of amount of norepinephrine and serotonin in brain. Another important amino acid present in tea is γ-aminobutyric acid (GABA) amount of which is increased during anaerobic treatment of fresh tea leaves and has potent role in hypertension (Horie and Kohata 2000). Studies of protein extraction or elimination may unbolt the possibility of simultaneous protein production from tea leaves as well as improvement of sensory attributes of tea. Few processes associated with theanine extraction have been tabulated in Table 4.

Extraction of polysaccharide from tea leaves and flowers

Attempt to extract carbohydrate from tea leaves or flower and search for its food and medicinal value are very new areas of research. It was found that cell wall of tea leaves and flowers contain water soluble polysaccharides which has immense health benefits like antioxidant, hypoglycemic, anti HIV, anti cancer, anti blood coagulant, anti radiation, and hepatoprotective (Quana et al. 2011; Nie and Xie 2011; Xu et al. 2012). Interestingly it was found that though UAE and MAE shows better polyphenol yield than the conventional one, conventional water assisted extract of carbohydrate from tea flower has more potential in vitro hypoglycemic activity than

carbohydrate obtained by UAE, MAE and enzyme assisted extraction (Wei et al. 2010). Acidic polysaccharide content in tea leaves and flowers was found to be highest by enzyme assisted treatment followed by boiled water extraction and then hot water assisted extraction. Enzyme content and temperature are the main parameters to be controlled for carbohydrate extraction. Protein denaturation may be caused during carbohydrate extraction from tea leaves and flower by enzymatic process (Wang et al. 2010). When tea leaves are considered, most literature enlightens about green tea polysaccharides, because degree of fermentation and various extraction conditions diminish carbohydrate content of black or oolong tea (Wang et al. 2012).

Adsorption assisted tea biomolecule separation from tea extract

Isolation of tea catechin is one of the major steps to be followed for crude tea extracts when required for food in pharmaceutical application. High Performance Liquid Chromatography, Capillary electrophoresis or High-Speed Countercurrent Chromatography are suitable methods for simultaneous isolation and determination for small amount of extract (Degenhardt et al. 2000) but commercialization of these processes for selective isolation of tea catechin is quite challenging. The application of theory of selective adsorption of polyphenols by activated charcoal, acid and alkali treated lignocellulosics or inorganic binder like resin as well as adsorption of caffeine by natural oligosaccharide like cyclodextrin and desorption by suitable mobile phase was found to be a good alternative for simultaneous large scale separation of tea biomolecules and decaffeination whenever needed. Use of adsorption process in different tea biomolecule separation has been tabulated in Table 5.

Conclusion

Optimization of tea polyphenol extraction is a complex mechanism. It involves chances of oxidation leading to degradation of these bioactive molecules. Simultaneous decaffeination when required raises further complication. Possibility of using low cost lignocellulosic adsorption has been described as a suitable method for large scale separation of tea component here in spite of large initial setup cost. Enzymatic treatment during tea extraction is recommended for improvement of sensory quality and degradation of caffeine which would be a feasible application of food biotechnology to optimize the yield of desired component from tea. This study also unlocks the option of choosing feasible environment friendly method to avoid food contamination with toxic components during extraction, facilitate yield, and enhance sensory, gustatory and medicinal property of tea extract by UAE, MAE, HPP, SFE, SWE and enzymatic treatment associated extraction than conventional extraction processes.

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