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Stability, antioxidant activity and in vitro bile acids-binding of green, black and dark tea polyphenols during simulated in vitro gastrointestinal digestion

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The stability of phenolic compounds and antioxidant activity, as well as the bile acids-binding activity of green, black, raw liubao and aged liubao tea during the in vitro gastrointestinal digestion were evaluated. After the in vitro gastrointestinal digestion, the total phenolic content of green tea remarkly decreased. However, it increased in the fermented tea of black, raw liubao and aged liubao. Meanwhile, the total catechin recovery of green tea was 52.79%, but the fermented tea of black, raw liubao and aged liubao were 100.27%, 92.73%, and 106.02%, respectively. After gastrointestinal digestion, the ABTS cation radical scavenging capacity of black tea and aged liubao tea increased, and the post-fermented liubao tea increased more significantly. In vitro bile acid binding by tea extracts of green, black, raw liubao and aged liubao tea binding of 17.92–43.55% bile acids. Therefore, teas have potential for hyperlipidemic prevention associated with cardiovascular disease.

Introduction

Recently, tea products have been observed an increasing interest in human life. The teas can be classified as unfermented tea (green tea), semi-fermented (oolong tea), fermented (black tea), and post-fermented (pu-erh tea, liubao tea) on the basis of the production methods. In the past decades, people found that tea products were effective for the prevention of various illnesses[1-3] . It had been reported that these effects were attributed to the polyphenol compounds in tea[4]. In general, the teas are digested in the gastrointestinal tract. The polyphenols compounds are released during the digestion and are absorbed in the intestinal tract to achieve this specific effect[5]. Tenore et al found that the polyphenol compounds can be decreased by average of 44.4% during the digestion, as well as 91.8% of native catechin in tea[6].

Bile acid biosynthesis in the intestinal tract plays an important role in maintaining cholesterol homeostasis. Potential cholesterol-lowering and cancer prevention ability of food fractions could be predicted by evaluating the ability of binding bile acid in vitro. Colestyramine is one kind of anticholesteremic agent, and is usually employed as the positive bile acids binding control in the previous studies[7-9]. Both in vitro and in vivo researches indicate that there is a positive correlation between the bile acid binding ability and cholestyramine dosage[10,11]. Binding of bile acid and enhancing the bile acid content in faeces have been assumed to be the possibility cholesterol-lowering mechanism of food fractions[7-9]. Gong et al found that the content of cholesterol and bile acid in the faeces of hyperlipidemia mice could be increased by 4.08-2.11 times by the adding of pu-erh tea theabrownin. It indicated that the theabrownin can improve the transformation and discharge of the cholesterol in food[12]. Recent researches demonstrated that phenolic substances in tea have the ability of binding bile acid. Ngamukote et al indicated that the content of bile acid could be reduced by the binding of gallic acid, catechin and epicatechin with taurocholic acid, glycodeoxycholic acid hydrate and turo-ursodesoxycholic acid, which thus reduced the solubility of cholesterol[13]. These studies indicate that polyphenols such as catechin, epicatechin, theaflavin and theabrownin are the main constituents affecting the concentration of the bile acid. Thus it can be seen that the polyphenols compounds have the potential to bind with the bile acid, which will consequently inhibit the absorption of cholesterol and increase the excretion of cholesterol and bile acid.

In our previous studies[14,15], we found that the extracts of liubao tea have the effect of reducing lipid and anticoagulation of the hyperlipidemic mice, and the major antihyperlipidemia individual catechin were finally identified. Our present study focused on the binding effect between the major active ingredients in tea (phenolic compounds) and the bile acids. Changes of the phenolic compounds and antioxidant activity were investigated before and after in vitro digestion. The correlations between the individual catechin and the bile acid binding ability were determined. All our studies showed the potential antihyperlipidemia ability of teas.

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Experimental

Chemicals

All reagents and chemicals used were either HPLC grade or analytical grade. The water was prepared using a compact ultrapure water system before use. Standards were: GA: (+)-gallic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). GC: (–)-gallocatechin; EC: (–)-epicatechin; EGCG: (–)-epigallocatechingallate; GCG: (–)- gallocatechin gallate; ECG: (–)-epicatechingallate were obtained from Institute of Beijing YingzeNaxin chemical technology (Beijing, China). Acetonitrile (HPLC grade) was bought from Merck Specialities Private Limited (Indian). Chemicals and reagents used to simulate the gastrointestinal digestion, and bile acid analysis, were: pepsin, pancreatin, glycocholic acid, glycochenocholic acid, glycodeoxycholic acid, taurochenocholic acid, taurodeoxycholic acid and cholestyramine, were purchased from Sigma-Aldrich (St. Louis, MO, USA). The bile acids analysis commercial kits (TBA testing kit, Shanghai Juchuang Biotech. Co. Ltd., (Shanghai, China). Folin–Ciocalteu's phenol reagent was bought from Coolaber science & technology (Beijing, China). L-Ascorbic acid (VC) was bought from Tianjing Bodi chemical industry CO., Ltd (Tianjing, China). 2,2,-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) was bought from Sigma–Aldrich (St. Louis, MO). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was bought from Tokyo Chemical Industry (St. Portland, Japan).

Preparation of extracts

Green tea and black tea samples were obtained from local markets in Nanning City of China as individual tea bags, raw liubao tea (Slightly fermented) and 3 years aged liubao (post-fermented) tea were obtained from China Tea CO., Ltd (Wuzhou, China). All teas were ground to powder. A total of 5.0000g powdered sample was mixed with boiled water (50 mL) and incubated at 80-85℃ for 5 min. After cooling, each sample extract was centrifuged, and supernatant was removed into a volumetric flask (50mL). An additional of deionized water was used to rinse out the centrifuge tube, which was vortexed and centrifuged as before. Supernatant was combined with the previous supernatant and dilute with deionized water to 50mL. The solution were frozen at -20℃ for further analysis.

Content of total polyphenols and catechins in tea

The total phenolic content was determined according to the International Standards Organisation (ISO) ISO 14502-1-2005E, by the Folin–Ciocalteu reagent. Briefly, 1 mL of sample extract was pipetted into a volumetric flask (100 mL) and mixed with distilled water to the mark. 1mL of the diluted sample was mixed with 5 mL of 10% Folin–Ciocalteu's phenol reagent. After 3-8 min, 4 ml of 7.5% sodium carbonate solution was added to the reaction mixture, then stand for 1 h before spectrometric analysis. The standard curves of various concentrations of gallic acid were used for quantification, and the results were expressed as mg of gallic acid per mL of sample extracts.

Analysis of individual catechins content by HPLC, and was performed according to the ISO 14502-2-2005E procedure. 1 mL of the sample extract was diluted to 10 mL with stabilizing solution (10% v/v acetonitrile with 500 μg/mL EDTA and ascorbic acid). Polyphenols were purified from the extracts after digestion by solid phase extraction (SPE) using ProElut C18 cartridges (Dikma Technologies, China). The purified solution was filtered through a 0.45 um nylon membrane filter and put into vials. An aliquot of 10 μL of the solution was injected into the HPLC system by an auto-sampler. The system, comprised of a Waters e2695 Separations Module, a Waters 2998 Photodiode Array Detector, with photodiode array detection at 278 nm, using a ZORBAX Eclipse Plus C18 column (250 mm×4.6 mm i.d., 5 μm) (Agilent Technologies, USA). Column temperature was set at 35 ℃. Mobile phase A, 9 % (volume fraction) acetonitrile, 2 % (volume fraction) acetic acid with 20 μg/mL EDTA. Mobile phase B, 80 % (volume fraction) acetonitrile, 2 % (volume fraction) acetic acid with 20 μg/mL EDTA. The flow rate of the mobile phase was 1 mL/min. The gradient of mobile phase A as follows: 0-10 min, 100%, 10-25 min, from 100% -68% and kept at this composition for 10 min. Then reset to 100% A and allowed to equilibrate 10min before the next injection. Catechin and gallic acid were identified by comparison of their retention times and spectra to those of the standards. Quantification of catechin and gallic acid was carried out by integrating the peak areas and using calibration curves. Results were expressed as microgram of each phenolic compound per 1 mL of sample.

Binding of bile acid

In vitro digestion procedure

The assay was performed according to a modification by Kahlon & Smith[9] as follows. The bile acid mixture (36 mmol/L) contained glycocholic acid (9 mmol/L), glycochenocholic acid (9 mmol/L), glycodeoxycholic acid (9 mmol/L), taurochenocholic acid (4.5 mmol/L) and taurodeoxycholic acid (4.5 mmol/L) in pH 6.8 phosphate buffer. This stock solution of was stored in the -20℃ freezer and diluted to the working solution (0.72 μmol/mL) before each assay. Three replicates of 1mL of green tea, black tea, raw liubao tea and aged liubao tea extract, cholestyramine 50 mg were tested. Four substrate blanks, one positive blank and three treatment replicates were weighed into 50mL screw-capped tubes. Samples were digested in 1 mL 0.01 mol/L HCl for 2h in a 37℃ shaker bath. After simulating gastric digestion, the sample pH was adjusted to 7-7.5 with 0.2 mol/L NaOH. Add 4 mL of bile acid mixture (0.72 μmol/mL) in a phosphate buffer, pH 6.8. A phosphate buffer (4 mL, pH 6.8) was added to the substrate blanks. Following by 5 mL of porcine pancreatin (8×USP, 10 mg/mL, in a Mphosphate buffer, pH 6.8) was added, tubes were incubated for 2 h in a 37℃ shaker bath. Mixtures were centrifuged at 6000rpm for 20 min at 25℃. Supernatant was removed into a second set of labeled tubes. Aliquots of pooled supernatant were frozen at -20℃ for bile acids analysis.

Bile acid analysis

The bile acids were measured colorimetrically with commercial kits. The bile acid binding activity was calculated as:

$$\textbf{Bile acid binding acitivity}\ (\%) = \frac{\boldsymbol{A}_{\textbf{mixture}-}\boldsymbol{A}_{\textbf{supernatant}}}{\boldsymbol{A}_{\textbf{mixture}}} \times \mathbf{100}$$

where Amixture is the bile acid concentration in the positive blank and Asupernatant is the bile acid concentration in the supernatant.

Antioxidant activity

Free radical (DPPH) scavenging assay

Experiments were carried out as a modification by Xiao et al.[16]. Briefly, 2.0 mL of test sample mixed with 2.0 mL of 0.2 mM DPPH that was dissolved in 100% ethanol. The mixture was shaken well and stand at room temperature in the dark. The absorbance of reaction mixture was recorded after 30 min at 517 nm. Vitamin C was used as a standard compound. The standard curves were acquired by plotting the DPPH scavenging of vitamin C (ranging from 1 to 25 mg/L). The results were calculated by Y (scavenging ratio) =-0.0452X (VC equivalents content)+1.1681 (R2=0.991). All samples were analyzed in triplicate.

ABTS cation radical scavenging capacity

The ABTS assay was developed by the reference of Du et al[17]. 10 mLof 7 mM ABTS+ solution was mixed with 178 μL of 140 mM potassium persulfate (K2S2O8) in the dark at room temperature for 13 h prior to use. The mixture was diluted with PBS buffer to an absorbance at 0.70 ± 0.05 at 734 nm. An aliquot of 0.1 mL of sample, blank (water) and standard mixed with 3.9 mL of diluted ABTS+ to react in the dark at room temperature, and absorbance was recorded at 734 nm after 5 min. Vitamin C was used as a standard compound. The standard curves were acquired by plotting the ABTS cation radical scavenging capacity of vitamin C (ranging from 1 to 25 mg/L). The results were calculated by Y (scavenging ratio) =-0.0037X (VC equivalents content)+0.5599 (R2=0.9989). All samples were analyzed in triplicate.

Statistical analysis

All data are presented as mean ± SD for three in vitro incubations that were analyzed in triplicate. The statistical analyses were performed using SPSS 19.0. Differences of $P<0.05$ were considered significant. Data were subjected to 1-way ANOVA, means compared using Tukey's test ($p<0.05$). Correlations among the antioxidant capacity, the total polyphenolic content and the bile acids-binding capacity were established using regression analysis at a 95% significance level. The correlations was considered significant when $P<0.05$.

Results and discussion

Changes of phenolic compounds

The phenolic compounds are characteristic constituents in tea, and they are also the main active ingredient in tea. Changes of total phenolic compounds in green, black, raw liubao and aged liubao tea extracts during the simulated in vitro gastrointestinal digestion were shown in Fig. 1. It can be seen that there was great difference of total phenolic compounds content between different kinds of tea samples. The total phenolic compounds content of green tea was higher than the other teas, and it was higher in liubao tea than that in black tea. Wu et al showed that the liubao tea have the highest tea polyphenol content than the other dark teas in China[18]. It indicated that the potential biological activity of liubao tea may be stronger than black tea though it belongs to post-fermented tea. The content of the total phenolic compounds in green tea (unfermented) was reduced by 6.12% after simulated digestion. However, content of the total phenolic compounds in black tea (fermented tea), raw liubao tea (fermented tea) and aged liubao tea (post-fermented) were increased by 10.06%, 3.11% and 26.86%, respectively. This indicated that the total phenolic compounds could be reduced in unfermented teas and be increased in fermented tea after the simulated in vitro gastrointestinal digestion. In addition, content of the total phenolic compounds increased with the increase of the fermentation degree. The decrease of the total phenolic compounds may be because of the degradation of catechins during the in vitro gastric and small intestinal digestion[19,20]. However, the phenolic polymers in fermented teas will be degraded during the gastrointestinal digestion, which thus produced more phenolic hydroxyl[21,22].

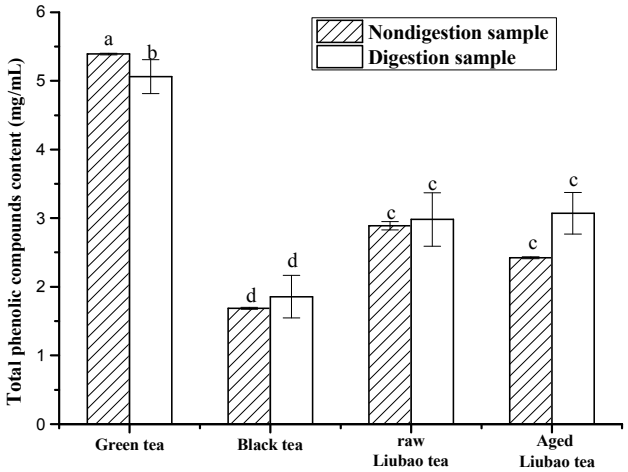

Fig. 1 Content of total phenolic compounds in green, black, raw liubao and aged liubao tea during the simulated in vitro gastrointestinal digestion. a–c Means between different kinds of tea samples after in vitro gastro-intestinal digestion with the same letter in row are not significantly different according to Tukey's multiple range test ($P > 0.05$).

Catechin content of tea samples

There was a positive correlation relationship between the catechin levels and tea radical scavenging activity. The high catechin levels may be responsible for the protective effects of tea in conditions related to oxidative stress, neoplastic transformations and cardiovascular disease[23,24]. Changes of catechin contents in green, black, raw liubao and aged liubao tea after simulated digestion were shown in Fig. 2-3 and Supplement 1. It indicated that total catechin recovery of green, black, raw liubao and aged liubao tea were 52.79%, 100.27%, 92.73% and 106.02%, respectively, after gastric intestinal digestion. It can be seen from Figs. 3 and 4 that the total catechin contents of green, black, raw liubao and aged liubao tea were 5042.82 μg/mL, 1478.08 μg /mL, 2253.76 μg /mL and 1803.77 μg /mL, respectively. The content of individual catechin was decreased the most in green tea after gastric intestinal digestion. However, content of individual catechin was more stable in fermented tea and increased with the increasing period of fermentation. After the digestion, individual catechin content of GA, GC, EC, EGCG, GCG, ECG were all decreased in green tea. GA, GC, EC, ECG were decreased and EGCG and GCG were increased in black tea. GA, GC, EGCG were decreased and EC, GCG and ECG were increased in raw liubao tea. Furthermore, GA, GC, EC, EGCG, GCG, ECG were all increased in aged liubao tea. Many studies have proposed that catechin will degrade during the in vitro simulated gastro-intestinal digestion[19,20], these studies have

shown that the catechins in both green and black teas will be obviously degraded at alkaline pH. This degradation isn't probably due to the decrease of antioxidant activity or total polyphenolic content, which maybe because of the formation of dimers. Our results suggested that the catechins of fermented tea water extracts will degrade and regenerate in gastrointestinal tract. This maybe attribute to the phenolic polymers that generated during the fermentation process. The phenolic polymers will be degraded further and individual catechins and other compounds will generate.

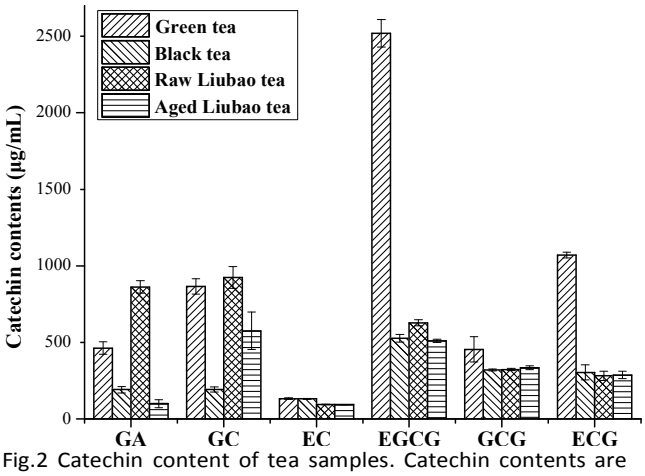

Fig.2 Catechin content of tea samples. Catechin contents are expressed as mean value (μg/mL tea extracts) ± SD (n = 3).

Antioxidant activity of tea extracts

The antioxidant activities of the tea extracts were usually expressed as Vitamin C Equivalent Antioxidant Capacity (VCEAC) in mg/mL. ABTS and DPPH are two kinds of stable radical species that usually employed for antioxidant activity measurements of the plant extracts[25,26]. They are commonly used independently to evaluate their efficacies. Aura et al indicated that the surviving phenolic compounds during the gastric-intestinal digestion were likely to reach the colon. These phenolic compounds will act as antioxidants or be bio-transformed into phenolic antioxidants and be absorbed in the large intestine/colon[27]. Our results as shown in Fig. 4 and Fig.5 indicated that the digestion products still possessed certain antioxidant activities and the order was Nondigestion sample ＞Digestion sample, except ABTS cation radical scavenging capacity of black tea and aged liubao tea.

DPPH radical scavenging activity of green, black, raw liubao and aged liubao tea showed a similar tendency during the in vitro gastro-intestinal digestion as shown in Fig. 4. The DPPH radical scavenging activity of green, black, raw liubao and aged liubao tea were decreased by 55.9%, 58.7%, 54.0% and 54.2%, respectively. It indicated that the DPPH radical scavenging activity of all tea extracts remarkly decreased after vitro gastro-intestinal digestion. This could be due to the fermenting degrees make no difference to the DPPH scavenging activity of tea, or it may be that the oxidation resultant products also have remarkable effect on the antioxidant activity[28].

ABTS cation radical scavenging capacity of green, black, raw liubao and aged liubao tea were shown in Fig. 5. During vitro gastro-intestinal digestion, ABTS cation radical scavenging capacity of green and raw liubao tea were decreased by 10.7% and 36.45%, respectively. However, ABTS cation radical scavenging capacity of black and aged liubao tea were increased by 22.9% and 26.0%, respectively. It could be speculated that the tea polyphenols polymerides structure will be changed and will be degraded during its transportation from intestine. The resultant small molecular phenolic compounds and breakdown products might have a higher ABTS cation radical scavenging capacity, which will directly contribute to the antioxidant capacity of the digestion samples. Mukai et al indicated that the increase of the radical scavenger activity was attributed to the deprotonation of the hydroxyl moieties present on the aromatic rings of the phenolic compounds[29]. The phenolic molecules structure will be changed during the transition from the stomach to the intestinal environment, which is attributed to the ionisation of the hydroxyl groups[30].

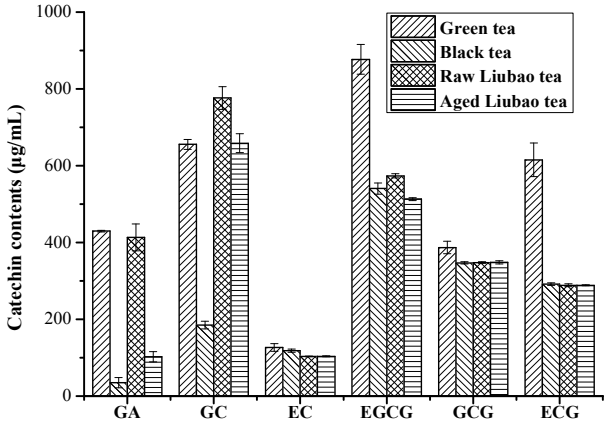

Fig. 3 Gastro-intestinal bioaccessibility of catechin from tea samples. Catechin contents are expressed as mean value (ug/mL tea extracts) ± SD (n = 3).

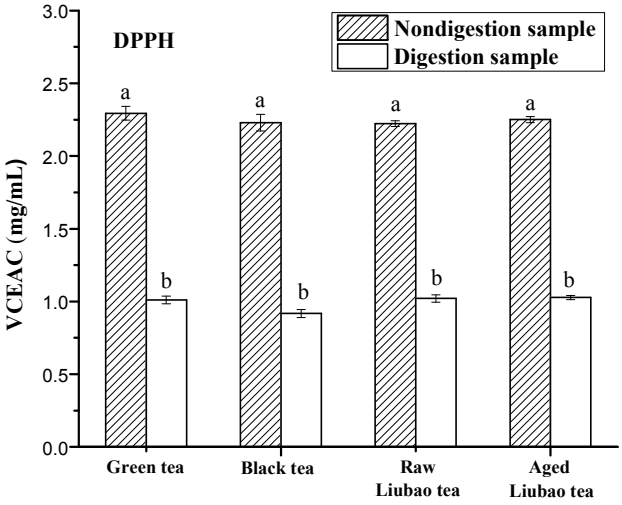

Fig. 4 Antioxidant activity (DPPH) of each sample (mg/mL extract) (mean ± SD, n = 3) after in vitro simulated gastro-intestinal digestion with the reference standards Vc. a–b Means between different kinds of tea products after in vitro gastro-intestinal digestion with the same letter in row are not

significantly different according to Tukey's multiple range test ($P > 0.05$).

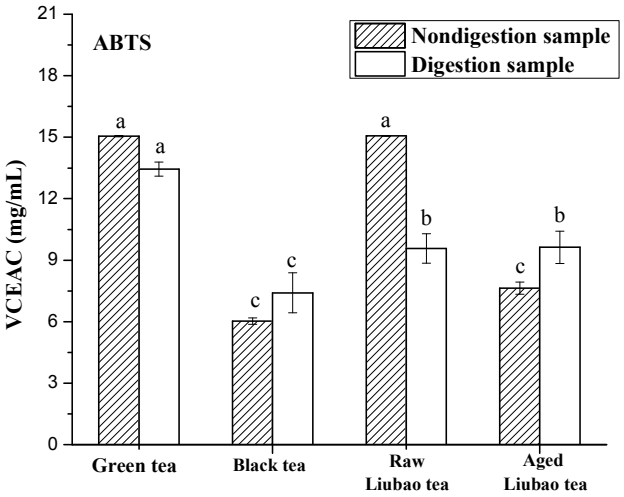

Fig. 5 Antioxidant activity (ABTS) of each sample (mg/mL extract) (mean ± SD, n = 3) after in vitro simulated gastro-intestinal digestion with the reference standards Vc. a–c Means between different kinds of tea products after in vitro gastro-intestinal digestion with the same letter in row are not significantly different according to Tukey's multiple range test ($P > 0.05$).

In vitro bile acid binding by tea extract

Cholestyramine is a bile acid sequestrant. It is positively charged non-digestible resins that bind to bile acids in the intestine to form an insoluble complex[31]. In our research, cholestyramine bound 95% of the bile acids. It is higher than the previous studies, Story & Kritchevsky found that 81% bile acid binding by cholestyramine using 50 mg of substrate and 50 µmol of bile acids[32]. It may be that the use of the higher substrate to bile acid ratio. In vitro bile acid binding by tea extracts on equal volume of tea extract was shown in Table1. Green, black, raw liubao and aged liubao tea bound 1.59, 0.46, 0.65 and 0.90 µM of bile acid per milliliter of sample, respectively, which was equal to 43.55, 12.54, 17.92 and 24.73 % of the total added BA. Assigning a bile acid binding value of 100% to cholestyramine (50mg), the relative bile acid binding on 1mL for the test samples of tea extract was green tea 45.83%, black tea 13.20%, raw liubao tea 18.86%, and aged liubao tea 26.03%. Bile acid binding for green tea was significantly higher than the other three teas. Relative bile acid binding on tea extract was green tea > aged liubao tea > raw liubao tea > black tea. The different bile acid binding may be associate with the phytonutrients (antioxidants, polyphenols, hydroxycinnamic acids, flavonoids, anthocyanins, flavonols, proanthocyanidins, catechins), hyrophobicity or active binding sites of the various tea[9]. On a tea extract, bile acid binding of 17.92–43.55% for green, black, raw liubao and aged liubao tea are very encouraging and could indicate health promoting benefits of these teas. Health properties of the food fractions were generally evaluated by testing their bile acid binding. Fat absorption, cancer causing toxic metabolites excretion and cholesterol utilization to synthesize more bile acids will be reduced by bile acids binding. It is supposed to be the lower cholesterol and prevent cancer mechanism by food fractions. Previous studies reported for fresh fruits and fresh green vegetables, blueberries 7%, plums 6%, prunes 5%, strawberries 5%, cherries 5%, cranberries 4%, apples 1%, broccoli 5%, mustard greens 4%, and potato cultivars as raw, steamed or steamed then cooled range from 20.55% to 36.50% under similar conditions[9,33]. It showed that bile acid binding capacity by tea extracts is significant higher than a variety of fruits and vegetables.

Table1. In vitro bile acid binding by tea extract on equal volume of tea extract[A]

Treatment	Bile acid binding		
	(µmol/mL tea extracts)	Percentage bile acid binding (%)	Relative to Cholestyramine (50mg), %
Green tea	1.59±0.25[a]	43.55±6.84[a]	45.83±7.20[a]
Black tea	0.46±0.02[b]	12.54±7.93[b]	13.20±8.34[b]
Raw Liubao tea	0.65±0.02[b]	17.92±5.30[b]	18.86±5.58[b]
Aged Liubao tea	0.90±0.02[b]	24.73±4.56[b]	26.03±4.80[b]

[A]Mean ± SEM within a column with different superscript letters differ significantly ($P \leqslant 0.05$), n =3.

Correlation analysis

Correlation analysis was employed to go to show the relationship among total polyphenolic content, scavenging activities and bile acids-binding capacity. The correlation analysis was measured for all samples during vitro gastro-intestinal digestion and was presented in Table 2. It indicated that the correlation between TP contents and DPPH activity was poor. This may be due to TP did not have the effect of the DPPH scavenging activity of tea[34]. It meant that TP contribute less to the DPPH antioxidant capacity of fermented teas. There was a positive correlation relationship between the individual phenolic compounds and the DPPH scavenging activity. This may be due to the structure changing of the phenolic compounds during the in vitro gastrointestinal digestion, and the newly produced compounds also contribute to the DPPH

Table 2 Correlation coefficient amongst total polyphenolic content, scavenging activities and bile acids-binding capacity

	TP	GA	GC	EC	EGCG	GCG	ECG	DPPH scavenging capacity	ABTS cation radical scavenging capacity
DPPH scavenging capacity	-0.16	0.320	0.173	0.11	0.353	0.046	0.248		
ABTS cation radical scavenging capacity	0.802*	0.819*	0.827*	0.04	0.612	0.593	0.626	0.367	
Bile acids-binding capacity	0.947*	0.042	0.571	0.300	0.924*	0.960*	0.923*	0.970*	0.561

*Significant at 5% level.

antioxidant capacity[19]. However, highly significant positive correlations of TP, GA, GC contents with ABTS cation radical scavenging capacity were observed. This result was similar to the earlier research that stated that TP content had correlation coefficients (r=0.836) with ABTS[35]. The other individual catechins also have positive correlations with ABTS cation radical scavenging capacity, but not significant. Chang et al indicated that the phenolic contents in plants contributed to antioxidant activity probably because of their redox properties, where the phenolic contents acted as reducing agents, hydrogen donors and singlet oxygen quenchers[36].

There was a significant positive correlation between TP, EGCG, GCG, ECG contents and bile acids-binding capacity. Significant positive correlation between DPPH scavenging capacity and bile acids-binding capacity were also observed. The other individual catechins also have positive correlations with bile acids-binding capacity, but not significant. Ikeda et al found that tea catechins rich in gallocatechin gallate and catechin gallate were better availability to decrease cholesterol absorption than that rich in epigallocatechin gallate and epicatechin gallate[37]. It can thus be seen that the esterifiable catechins have higher bile acids-binding capacity than the unesterified catechins. This was in accordance with our previous results.

Conclusions

The tea has a positive function in health promotion. Phenolic polymers could be degraded and produce individual catechins and some other compounds during the in vitro gastrointestinal digestion of fermented tea extracts. These individual catechins have large contribution to ABTS cation radical scavenging capacity, and the interesting unknown compounds deserve further exploration. The tea extracts possess excellent antioxidant capacity and in vitro bile acid binding capacity, where the liubao tea show more advantage than the other fermented tea. Fermented tea like liubao tea has a wider space in the functional research field, which requires more in-depth research in future.

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