Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 101 (2006) 1178–1182
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase

Qiang He *, Yuanping Lv, Kai Yao

College of Light Industry and Food Engineering, Sichuan University, Chengdu 610065, PR China

Received 30 November 2005; received in revised form 7 January 2006; accepted 10 March 2006

Abstract

Tea polyphenols (TP) possess many beneficial properties, such as reducing the risk of cancer and heart diseases, and acting as natural antioxidants for the food industry. At the same time, tea polyphenols might inhibit digestive enzymes and reduce food digestibility. To explore this possible antinutritional property, the effects of tea polyphenols on the activity of four typical digestive enzymes were investigated. HPLC analysis of the tea polyphenols extracted from Chinese green tea indicated that their catechin content was 93.6% (w/w), and that the content of ester bond-containing polyphenols was more than 82%. Measurement of the interaction of gelatin with tea polyphenols was first carried out, in order to model enzyme protein–TP interaction. It proved that tea polyphenols were capable of binding and precipitating protein, suggesting a potential ability of TP to denature digestive enzymes. In addition, the inhibitory effects of tea polyphenols on α-amylase, pepsin, trypsin and lipase were studied. In the presence of 0.05 mg/ml tea polyphenols, the inhibition ratios of α-amylase, pepsin, trypsin and lipase were, respectively, 61%, 32%, 38% and 54%, suggesting that TP might possess antinutritional properties.

Keywords: Tea polyphenols; α-Amylase; Pepsin; Trypsin; Lipase

1. Introduction

Tea is the second most common beverage in the world next to water, and tea drinks are popular worldwide. Many epidemiological studies (Azam, Hadi, Khan, & Hadi, 2004; Kuroda & Hara, 1999) have shown that regular consumption of tea is associated with reduced risk of several forms of cancer. For example, regular drinking of green tea protects against lung and skin cancer and heart diseases. These protective effects are often attributed to the tea polyphenols (TP), in particular, the catechins. The primary catechins in TP include (±)-catechin (C), (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epicatechin (EC), as shown in Fig. 1. Further studies (An et al., 2004; Cai et al., 2002) demonstrated the antibacterial and antioxidative activities of TP, showing good prospects for their use as preservatives and antioxidants. TP exhibits strong free radical scavenging ability in both aqueous- and lipid-phase assay systems, and in some cases tea polyphenols are up to five times more effective than vitamin C or vitamin E. In fact, TP has been employed as a natural antioxidant in the food industries of many countries.

On the other hand, TP plays an important role in protein precipitation and enzyme inhibition, through forming various complexes (Cartriona, Cai, Russell, & Haslam, 1988; Shi, He, & Haslam, 1994). It is known that most polyphenols, such as tannic acid, gallotannin, catechin and proanthocyanidin, can react with proteins, which should result in the formation of sediment and haze. In food industry this phenomenon occurs frequently in the production of beverages, such as beers, fruit and vegetable juices, leading to low quality products. TP exhibits strong complexing abilities with enzymes. This should inevitably result in the change of enzyme molecular configuration

* Corresponding author. Tel.: +86 28 85404298; fax: +86 28 85405237.
E-mail address: heq361@163.com (Q. He).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.03.020

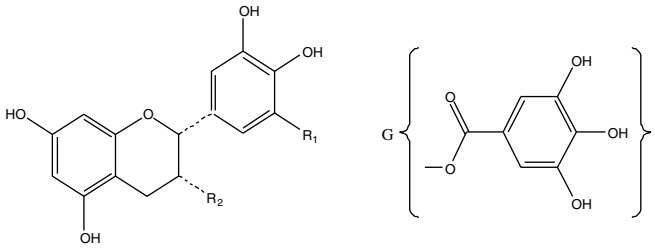

Fig. 1. Structures of major polyphenols present in green tea [EGCG: R_1 is OH, R_2 is galloyl group (G); ECG: R_1 is H, R_2 is G; EC: R_1 is H, R_2 is OH; EGC: R_1 and R_2 are OH].

and lead to the loss of catalytic activity. Many enzymes, such as tyrosinase, peroxidase, trypsin (Huang, Kwok, & Liang, 2004), decarboxylase (Bertoldi, Gonsalvi, & Voltattorni, 2001), squalene epoxidase (Abe et al., 2000) and ribonuclease (Ghosh, Maiti, & Dasgupta, 2004), were found to be denatured by tea polyphenols. So it could be speculated that TP should bind and precipitate digestive enzymes and thereby reduce food digestibility, when excessively.

Usually, TP is considered to be a safe natural product. However, it might act as an antinutritional factor, in terms of the inhibition of enzymes, when ingested in excess. To minimize the antinutritional effects and make full use of tea polyphenols in the food industry, knowledge of the interaction between TP and digestive enzymes is desirable. In this study, tea polyphenols were extracted from Chinese green tea and their inhibitory effects on four typical digestive enzymes was investigated. Although the effects were assayed in vitro, the results of this work should be relevant to the human body.

2. Materials and methods

2.1. Materials

Fresh commercial Chinese green tea was used for the extraction of polyphenols. The catechin samples employed for HPLC analysis include C. EC, EGC, ECG, GCG and EGCG, and were purchased from Sigma. α-Amylase, pepsin, trypsin and lipase were purchased from Mingzhu Chemical Co. (Shanghai, China). All other chemicals were of the highest purity available.

2.2. Extraction and determination of tea polyphenols

The polyphenols were isolated from fresh commercial Chinese green tea, as described before (Liang, Huang, & Kwok, 1999). The tea was first refluxed with water. After filtration the tea liquid was concentrated in a rotary evaporator and then chloroform was added to remove caffeine, lipids and chlorophyll. The aqueous phase was extracted with-ethyl acetate and the ethyl acetate phase was freeze-dried.

The catechins content of the dried TP sample was determined by HPLC with a DAD detector using a Hypersil ODS C18 column (4.6 × 250 mm, 5 μm). The mobile phase was a mixture of A (aqueous solution of 0.2% CH_3CN) and B (CH_3OH). During the first 12 min, B was increased from 0% to 50%, and from 13 min to 20 min it was increased to 100%. The injection volume was 20 μl and the flow rate was 1.2 ml/min. Detection was set at 280 nm. Standard curves of peak area, as a function of the concentrations of catechins, were prepared for quantitative analysis.

2.3. Interaction of tea polyphenol with gelatin

Reactions took place in 10 ml volumetric flasks. TP was dissolved in water to 0.05 mg/ml, 0.10 mg/ml and 0.15 mg/ml. Gelatin solutions in water, whose concentrations varied from 0.02 mg/ml to 0.16 mg/ml were prepared. TP and gelatin solutions were mixed quantitatively in flasks under shaking. After standing for 24 h at 25 °C, the mixture was centrifuged (MSE centrifuge, Model GF-8, 3000 rpm, 20 min) and the suspended substance (reaction products) was removed. The supernatant was analyzed at 280 nm by UV spectrophotometer (UV-2501PC, Japan). All reactions were carried out in triplicate.

2.4. Interactions of tea polyphenols with α-amylase, pepsin, trypsin and lipase

The general procedure for studying the interaction of TP with enzyme is to mix solutions of the two substances, incubate for an appropriate time, and to determine the enzyme activity in the mixture after reaction termination by quickly decreasing the temperature to 4 °C. The control enzyme activity is determined by the same procedure described above but without TP added. The inhibition of enzyme activity in the presence of TP is calculated as follows:

$$\text{inhibition } (\%) = [1 - (\text{activity test}/\text{activity control})] \times 100.$$

The enzyme assays were performed as described previously (Abuereish, 1996; Wang, 2005). In brief, α-amylase was assayed in a reaction medium (100 μl, pH 6.9) containing 1.0 mM Na_2HPO_4, 6.0 mM NaCl, 0.6 mM H_3PO_4, 0.38 mg/ml soluble starch and 9.0 μg/ml α-amylase. Pepsin assay was performed in a reaction medium (100 μl, pH 2.0) containing 1.0 mM NaOAc, 48 mM HCl, 0.025 mg/ml haemoglobin (Sigma) and 6.0 μg/ml pepsin. Trypsin was assayed in a reaction medium (100 μl) containing 40 mM Tris (pH 7.9), 7.0 mM NaCl, 0.38 mg/ml BAEE (Sigma) and 12 μg/ml trypsin. Lipase assay was performed in a reaction medium (100 μl) containing 31 mM Tris–HCl (pH 5.0), 0.05 mg/ml olive oil, 0.25 mg/ml polyvinyl alcohol and 11 μg/ml lipase. All the assays were conducted in the presence or absence of tea polyphenols (TP, 0.05 mg/ml). TP were dissolved in distilled water and added to the reaction mixtures just before starting the reaction. As a control, equal volumes of distilled water without TP were added. All samples were analyzed in triplicate.

3. Results and discussion

3.1. Determination of tea polyphenols

The HPLC detection and quantitative analysis, as shown in Fig. 2 and Table 1, indicated that the content of catechins in the dried TP sample was 93.6%. It contained six main catechins, namely (±)-catechin and (−)-epigallocatechin (C and EGC, 5.1%), (−)-epigallocatechin gallate (EGCG, 40.9%), (−)-epicatechin gallate (ECG, 30.4%), (±)-gallocatechin gallate (GCG, 10.9%) and (−)-epicatechin (EC, 6.3%). A little caffeine (elution time 10.8 min) was found in the TP sample. In comparison with green tea infusions, the extracted TP has an analogous composition and proportion of catechins.

The catechins present in TP exhibit different biological properties, due to the differences in their molecular structure. It is reported that the more galloyl groups the catechin contains, the stronger is its ability to scavenge free radicals (Cai et al., 2002; Dreosti, 2000). On the other hand, the ester bond-containing catechins, such as EGCG and ECG, possess greater ability to inhibit enzymes (Bertoldi et al., 2001). EGCG has been the focus of many studies because of its prominent biological functions such as inhibition of enzymes, and inhibition of cell proliferation and tumour angiogenesis. The EGCG, ECG and GCG account for more than 82% (w/w) of the TP sample. It is therefore speculated that TP will take part in complexing reactions with protein and, to some extent, affect the activities of digestive enzymes.

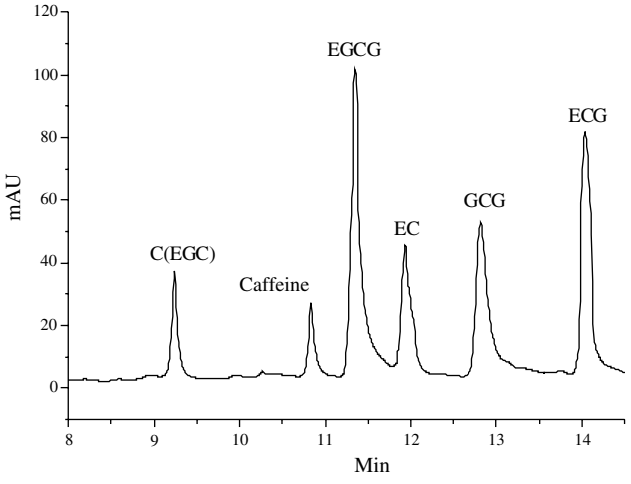

Fig. 2. HPLC chromatograms of green tea extracts (mobile phase: A = aqueous solution of 0.2% CH_3CN, B = CH_3OH; column: Hypersil ODS C_{18}, 4.0 × 150, 5 μm; flow rate: 1.2 ml/min; detector: DAD, 280 nm; injection volume: 20 μl; gradient: 0–12 min with 0–50% B, 13–20 min with 50–100% B).

Table 1
The quantitative analysis of the main catechins in the TP extract

Composition	Equation of standard curve[a]	R^2	Content (%, w/w)
C(EGC)	$Y = 8.1066X + 23.866$	0.9997	5.1
EGCG	$Y = 11.603X - 330.99$	0.9993	40.9
ECG	$Y = 15.083X + 14.155$	0.9800	30.4
GCG	$Y = 14.402X + 9.1619$	0.9830	10.9
EC	$Y = 8.6739X - 13.361$	0.9995	6.3

[a] Y is the peak area (mAU × s) and X is the concentration of catechin (mg/l).

3.2. Interaction of tea polyphenol with gelatin

Considering the fact that enzymes are protein in nature, the interaction of tea polyphenols with protein was first investigated, to explore their complexing ability with enzymes. As a typical protein, gelatin (molecular weight 100 kDa) was employed in the investigation. The content of catechins in aqueous solution containing TP and gelatin can be characterized by measuring the absorbance value of catechins at 280 nm, where gelatin has a negligible absorbance (Shi et al., 1994). The absorbance should decrease when some of catechins were precipitated together with gelatin during the reaction. Supposing that a TP solution with fixed concentration has an initial absorbance, A_0, at 280 nm and that after interaction with gelatin the absorbance decreases to A, the relative absorbance could be defined by $RA = (A_0 - A)/A_0$. Hence, a higher value of RA means less catechins remaining in solution, or more catechins have been precipitated with gelatin, i.e., increased TP–gelatin interaction has taken place. The bonding capacity of TP with protein can therefore be evaluated by the values of RA obtained.

Fig. 3 reflected the precipitation ratios of gelatin by tea polyphenols (expressed as RA). From the results, it is clear that the TP–gelatin interaction occurs easily even in dilute solution. When the concentration of gelatin is 0.02 mg/ml, 22% of gelatin in solution was precipitated by 0.05 mg/ml of tea polyphenols. With the increase of gelatin and TP concentrations, the bonding ability of TP with gelatin was strengthened. The precipitation ratio was up to 84% when the concentrations of gelatin and TP were 0.16 mg/ml and 0.15 mg/ml, respectively. Based on these observations, it could be deduced that tea polyphenols can precipitate pro-

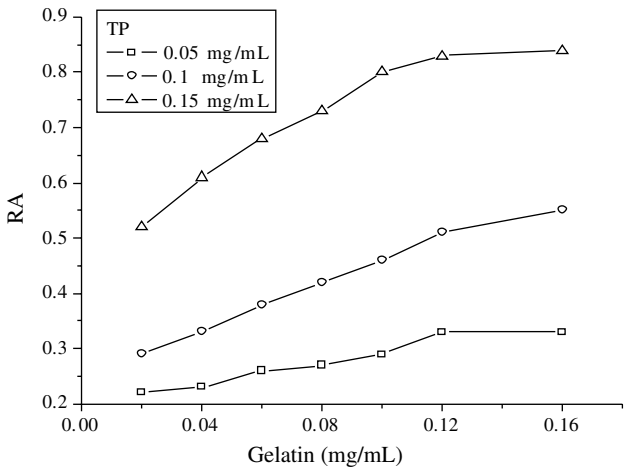

Fig. 3. RA value of the interaction between TP and gelatin (measurements were made at 25 °C).

tein and have the potential to inhibit the activities of enzymes.

3.3. Inhibition of α-amylase, pepsin, trypsin and lipase by tea polyphenols

Four typical digestive enzymes including α-amylase, pepsin, trypsin and lipase, were used to investigate the TP–enzyme interaction and to explore the potential antinutritional property of tea polyphenols. In experiments, tea polyphenols showed different bonding ability in vitro with the digestive enzymes employed. As shown in Table 2, the inhibition ratios of α-amylase, pepsin, trypsin and lipase were 61%, 32%, 38% and 54%, respectively, when the TP concentration was 0.05 mg/ml. These inhibitory effects should be reinforced by an increased TP concentration. Hence, there could be a reduction of the digestibilities of carbohydrates, proteins and lipids, whose hydrolyzation reactions in the gut are enzyme mediated. Hence, TP might act as an antinutritional factor, in terms of its potential to inhibit the activities of digestive enzymes.

It is well known that molecular weight plays an important role in the interaction between macromolecular compounds. Of the enzymes tested, tea polyphenols showed the strongest inhibition of α-amylase which has the highest molecular weight. In the presence of 0.05 mg/ml of TP, α-amylase had the highest loss of enzyme activity and the residual activity is only 39% of the original. However, in comparison with pepsin, trypsin has a smaller molecular weight but a higher activity loss, suggesting that the inhibitory effects of tea polyphenols on enzymes do not completely depend on the molecular weight of the enzyme. In a recent report (Tagliazucchi, Verzelloni, & Conte, 2005), it was found that some polyphenols could enhance the enzymatic activity of pepsin, which is different from the results obtained herein. This might result from the differences between polyphenols and the different of pepsin sources, which should be further studied. The observed inhibitory effect of TP on lipase activity is in agreement with the result of a recent study (Nakai et al., 2005) where the effect of oolong tea polyphenols on pancreatic lipase was investigated.

The main mechanism of TP–protein (TP–enzyme) bonding is considered to be non-covalent interactions (Dreosti, 2000; Siebert, Troukhanova, & Lynn, 1996). Tea polyphenols contain hydroxyl groups and galloyl groups in their molecular structure. The phenolic groups can form hydrogen bonds with the polar groups (amide, guanidine, peptide, amino and carboxyl groups) of protein. In other words, the composition and quantity of the polar groups in the enzyme protein will affect the formation and stability of hydrogen bonds between TP and the enzyme. On the other hand, the galloyl groups in TP exhibit certain hydrophobicity, which was well discussed in our previous report (He, Shi, & Yao, 2006). With the recognition that there are many hydrophobic amino acids present in enzyme protein, such as proline, phenylalanine and tyrosine, it could be considered that tea polyphenols should strongly bind enzymes through hydrophobic association. The occurrence of hydrogen bond and hydrophobic association will change the enzyme molecular configuration, resulting in an impact on the enzyme activities. So, it could be concluded that tea polyphenols might act as an antinutritional factor, in terms of their inhibitory effects on digestive enzymes, which may be due to the cooperative effects of hydrophobic association and hydrogen bond formation between TP and the enzymes.

Table 2
Inhibition of enzymes by TP (0.05 mg/ml)

Enzymes	Molecular weight ($\times 10^3$)	Isoelectric point	Inhibition (%)
α-Amylase	77	5.2–5.6	61
Pepsin	36	1.5–1.7	32
Trypsin	25	7.0–7.5	38
Lipase	51	6.0–6.2	54

Acknowledgement

The authors thank the Natural Science Foundation of China for the financial support of this work (20472058).

References

Abe, I., Seki, T., Umehara, K., Miyase, T., Noguchi, H., Sakakibara, J., et al. (2000). Green tea polyphenols: novel and potent inhibitors of squalene epoxidase. *Biochemical and Biophysical Research Communications, 268*, 767–771.

Abuereish, G. M. (1996). Pepsin inhibitor from roots of *Anchusa strigosa*. *Phytochemistry, 48*, 217–221.

An, B. J., Kwak, J. H., Son, J. H., Park, J. M., Lee, J. Y., Jo, C., et al. (2004). Biological and anti-microbial activity of irradiated green tea polyphenols. *Food Chemistry, 88*, 549–555.

Azam, S., Hadi, N., Khan, N. U., & Hadi, S. M. (2004). Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate: implications for anticancer properties. *Toxicology In Vitro, 18*, 555–561.

Bertoldi, M., Gonsalvi, M., & Voltattorni, C. B. (2001). Green tea polyphenols: novel irreversible inhibitors of dopa decarboxylase. *Biochemical and Biophysical Research Communications, 284*, 90–93.

Cai, Y. J., Ma, L. P., Hou, L. F., Zhou, B., Yang, L., & Liu, Z. L. (2002). Antioxidant effects of green tea polyphenols on free radical initiated peroxidation of rat liver microsomes. *Chemistry and Physics of Lipids, 120*, 109–117.

Cartriona, M. S., Cai, Y., Russell, M., & Haslam, E. (1988). Polyphenol complexation – some thoughts and observations. *Phytochemistry, 27*, 2397–2409.

Dreosti, I. E. (2000). Antioxidant polyphenols in tea, cocoa, and wine. *Nutrition, 16*, 692–694.

Ghosh, K. S., Maiti, T. K., & Dasgupta, S. (2004). Green tea polyphenols as inhibitor of ribonuclease A. *Biochemical and Biophysical Research Communications, 325*, 807–811.

He, Q., Shi, B., & Yao, K. (2006). Interactions of gallotannins with proteins, amino acids, phospholipids and sugars. *Food Chemistry, 95*, 250–254.

Huang, H. H., Kwok, K. C., & Liang, H. H. (2004). Effect of tea polyphenols on the activities of soybean trypsin inhibitors and trypsin. *Journal of the Science of Food and Agriculture, 84*, 121–126.

Kuroda, Y., & Hara, Y. (1999). Antimutagenic and anticarcinogenic activity of tea polyphenols. *Mutation Research, 436*, 69–97.

Liang, H. H., Huang, H. H, & Kwok, K. C. (1999). Properties of tea-polyphenol-complexed bromelain. *Food Research International, 32*, 545–551.

Nakai, M., Fukui, Y., Asami, S., Toyoda-Ono, Y., Iwashita, T., Shibata, H., et al. (2005). *Journal of Agricultural and Food Chemistry, 53*, 4593–4598.

Shi, B., He, X. Q., & Haslam, E. (1994). Gelatin–polyphenol interaction. *Journal of American Leather Chemists Association, 89*, 98–104.

Siebert, K. J., Troukhanova, N. V., & Lynn, P. Y. (1996). Nature of polyphenol–protein interactions. *Journal of Agriculture and Food Chemistry, 44*, 80–85.

Tagliazucchi, D., Verzelloni, E., & Conte, A. (2005). Effect of some phenolic compounds and beverages on pepsin activity during simulated gastric digestion. *Journal of Agricultural and Food Chemistry, 53*, 8706–8713.

Wang, Z. (2005). *Methods for the analysis of enzyme activity*. *Food enzymology*. Beijing: Light Industry Press of China.