MITIGATION OF INHIBITORY EFFECT FOR A-AMYLASE AND A-GLUCOSIDASE BY GREEN TEA WITH FOOD ADDITIVES, OOLONG TEA, AND BLACK TEA

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ABSTRACT

The purpose of present study was to investigate the effects of extracts of green tea and additives, oolong tea, and black tea on α-amylase and α-glucosidase activities *in vitro.* As part of the characterisation of such foods, inhibition of alpha (*α*)-amylase and *α*-glucosidase are used to assess components for their potential ability to modify the post-prandial glycaemic response. The results of the both enzyme inhibition activity were found in a concentration and extraction time-dependent. The values for oolong (wūlóngchá) with 2.5 min/50 ml and 5.0 min/50 ml were 39.5% and 64.3% on 1.5 g/100 ml, respectively, as compared with acarbose as positive control compound. The values for black tea with 2.5 min/50 ml and 5.0 min/50 ml were 63.2% and 84.9% on 1.5 g/100 ml, respectively. The IC_{50} for α-amylase of green tea ranged from 44.9 to 79.1 ug/ml. Among analyzed extracts, green tea + honey was the lowest α-amylase inhibition activity (IC_{50} was 404.5 ug/ml on 2.5 min. and IC_{50} was 204.0 ug/ml on 5.0 min.). The IC_{50} for α-glucosidase of green tea ranged from 117.9 to 125.9 ug/ml. Among analyzed extracts, green tea + honey was the lowest α-glucosidase inhibition activity (IC_{50} was 667.6 ug/ml on 2.5 min. and IC_{50} was 334.2 ug/ml on 5.0 min.). All extract from green tea with additives, oolong tea, and black tea possess moderate α-amylase inhibition with potent α-glucosidase inhibitory activity.

KEYWORDS: α-amylase, α-glucosidase, black tea, green tea, oolong tea

INTRODUCTION

Tea (*Camellia* L.) is the most widely-consumed beverage or medicine in the world. Black, Green and Oolong teas are all produced from the hybrids with an affinity to three key taxa of the genus *Camellia* L. It is an important economic crop of the warm temperate and subtropical forests of that region. The biochemical components of tea leave include polyphenols (catechins and flavonoides), alkaloids (caffeine, theobromine, theophylline, etc.), volatile compounds, polysaccharides, amino acids, lipids and vitamins show a variety of bioactivities. In this context, several studies have revealed its health benefits and medicinal potentialities for several ailments.

Green tea, oolong tea, and black tea are derived from leaves of the *Camellia* L., but different manufacturing process result in tastes and flavors of each product as well as their functions (Uchida et al., 2013). Green tea is classified as non-fermented tea, oolong tea as semi-fermented tea, and black tea as fermented tea, based on the differences in the degree of fermentation during manufacturing. During tea fermentation, the bioactive polyphenols such as flavan-3-ols in tea leaves undergo polyphenol oxidase-dependent oxidative polymerization, resulting in the formation of theaflavins and thearubigins (Lin et al., 2003).

Tea is a natural and healthy drink, but commercial products marketed as tea can contain a wide range of different additives. Evaluation of the health effects of nonnutritive dietary substances is complicated for many reasons. It is necessary to consider both average and peak exposures, the potency or level of activity of the substances, and the quality of the experimental and epidemiologic data.

Alpha (α)-amylase is both a component of saliva and secreted from the pancreas as an endohydrolase that cleaves the internal α-(1,4) bonds of starch into shorter, linear and branched dextrin chains. The resultant dextrin mixture is then further hydrolyzed into glucose by α-glucosidase, located on the microvilli brush border of the intestine (Ren, 2011). α-amylase (E.C.3.2.1.1) is the major digstive enzyme in saliva. It catalyzes hydrolysis of the α-1,4-glucosidic linkage of starch and split starch components such as amylose and amylopectin into smaller oligosaccharides, including maltose (Ismail et al., 2018). The efficiency of mastication is important for salivary amylase to penetrate the food bolus. Polyphenols are known to inhibit the activity of digestive enzymes such as α-amylase and α-glucosidase, leading to a decrease in post-prandial hyperglycemia (Bailey et al., 2001).

Alpha-glucosidases (E.C.3.2.1.20) hydrolyzes terminal non-reducing (1→4)-linked α-glucose residues to release a single alpha-glucose molecule. The enzyme is a carbohydrate-hydrolase that releases alpha-glucose as opposed to beta-glucose. Beta-glucose residues can be released by glucoamylase, a functionally similar enzyme. α-Glucosidase inhibitors are drugs that inhibit glucosidases in the intestine and suppress postprandial elevation of plasma glucose by delaying the digestion and absorption of carbohydrates (Nemoto et al., 2011; Griffith, 2012). The overall effect of glucosidase inhibition also reduces the occurrence of insulin resistance, thereby preventing further insulin-dependent disorders (Jones et al., 2011).

This present work aims to determine the effects of several commercial products of tea and additives on the inhibition of α-amylase and α-glucosidase using experimental amylase and glucosidase inhibition assays.

MATERIALS AND METHODS

Sample extract

The samples for analysis were six products of popular green teas (accordingly to the statistics of Korean tea markets). Daily method of tea making (household preparation) from the stock of green teas has been used to prepare aqueous extracts. The aqueous extract is prepared in ratio 1.5 g : 50 ml or 1.5 g : 100 ml with the consideration of the absorption volume of green tea leaves. The time to boil tea was doubled to five minutes, compared with 2.5 minutes. Tea is put into tea pottery for times (2.5 min. or 5.0 min.) and cooled down for 30 min.

α-amylase inhibitory assay

The determination of α-amylase inhibitory activity was carried out by quantifying the reducing sugar (maltode eqivalent) liberated under assay conditions by the method described Apostolidis et al. (2007) with some modification. The aasy mixture containing 25 µl of 50 mM phosphate buffer pH 6.8, 2.5 µl extract and pre-incubated porcine α-amylase (0.25 U/ml) were incubated at 37°C for 10 min. After pre incubation, 25 µl of 0.5% starch solution was added. The reaction mixtures were then incubated at 37°C for 10 min. The reaction was terminated with the addition of 150 µl of 90 mM 3,5-dinitrosalicylic acid (DNS) reagent and placed in boiling water bath for 10 minutes. The extract was then cooled to room temperature until use. Absorbance (A) was measured at 540 nm. Acarbose (4",6"-Dideoxy-4"-([1S]-[1,4,6/5]-4,5,6-trihydroxy-3-hydroxymethyl-2-yclohexenylamino)-maltotriose) (Sigma Aldrich Chemical Co,

USA) was used as reference standard (positive control). Control incubations represent 100% enzyme activity and were conducted in a similar way by replacing extracts with vehicle. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. Separate incubation carried out for reaction t = 0 was performed by adding samples to DNS solution immediately after addition of the enzyme. The concentration of the extract required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated by regression analysis. Experiments were performed in triplicate.
Percent α-amylase inhibition was calculated as follows: (1–B/A) × 100, where A is the absorbance of control and B is the absorbance of samples containing extracts.

α-glucosidase inhibitory assay
The bioassay method of multiwell plate system was applied for α-glucosidase inhibitory activity assay as described by Deutschlander et al. (2009) with some modification. Extracts and catechins were prepared as described above. The test compound and 2 mU of Yeast α-glucosidase (Cat. No: G 5003, Sigma Aldrich Chemical Co, USA) was dissolved at a concentration of 0.1 U/ml in 100 mM sodium acetate buffer (pH 5.6). Enzyme source was prepared bovine serum albumin 2000 mg/ml and sodium azide 200 mg/ml in 100 mM sodium acetate buffer (pH 5.6). Paranitrophenyl-α-D-glucopyranoside (pNPG) (Cat. No: N 1377, Sigma Aldrich Chemical Co, USA) was used as substrate. A total of 20 ul from each extract were diluted to 97 µL in 0.1 M sodium acetate buffer (pH 5.6) and pre-incubated in 96-well plates at 37°C for 10 min. The reaction was initiated by adding 3 µL of 3 mM pNPG as substrate. The plate was incubated for an additional 10 min at 60°C, followed by addition of 100 µL 1 M NaOH to stop the reaction. All test compounds were prepared in DMSO as described above. The final concentrations of extracts and catechins were between 0.03–10 µg/mL and 5–1000 µM, respectively. The final concentration of α-glucosidase was 20 mU/mL. The optical density (OD) of the solution was read using the Microplate Reader (VersaMax, Califonia, USA) at the wavelength 405 nm. The reaction system without tea extracts was used as control and system without α-glucosidasewas used as blank for correcting the background absorbance. Acarbose was used as reference standard (positive control). Acarbose, known as BAY g 5421, is an α-glucosidase inhibitor that prevents absorption of sucrose and maltose. All samples were prepared in triplicate.

The inhibitory concentration of the extract required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated by regression analysis.

Inhibition of free radical scavenging activity was calculated using the following equation.
Inhibition (%) = 100 × (absorbance of the control - absorbance of the sample)/ absorbance of the control.

The ability of the extracts to scavenge at 50% of the α-amylase and α-glucosidase, IC_{50} was determined from the graph plotted in GraphPad Prism software. Regression analysis by a dose response curve was plotted to determine the IC_{50} values.

Statistical Analyses
All assays were carried out in triplicate and the values presented are the average of three replicates. The results were expressed as the mean±SD. Statistical analyses of the differences between samples were carried out by one-way analysis of variance (ANOVA), followed by post hoc multiple comparisons with Duncan's test and student's t-test with the PASW (Predictive analytics software) statistics package for Windows program. Differences were considered significant if the *p*-value was less than 0.05.

Table 1. The information of tea and additives in this study

Type	Green tea extract	Additives	Product name	**Country**
Green tea	100%	-	Osulloc	Jeju province, Korea
Green tea + brown rice	30~40%	Brown rice: 60~70%	Simplus	Pocheon, Gyeonggi province, Korea
Green tea + lemon	93%	7%	Tetley Green Tea Lemon	United Kingdom
Green tea + honey	?	Honey Lemon Chamomile	Lipton, Green tea Honey Lemon Chamomile	USA.
Oolong tea (Wūlóngchá)	100%	-	Gong Fu Tea|chA Episode 13	China
Black tea	100%	-	Assam Black tea	India

RESULTS

α-amylase inhibitory effects

In this study, the inhibitory effects of six extracts against α-amylase were investigated. The percentage inhibition at 1.5 g/50 ml and 1.5 g/100 ml concentrations of six extracts including green tea showed a concentration-dependent reduction in percentage inhibition (Table 2). The high α-amylase inhibitory found on black tea extracts. The low α-amylase inhibitory found on green tea + honey extracts. The inhibition of α-amylase by extraction time on 2.5 minutes showed no significant difference from one another on 5.0 minutes. However, inhibitory activities for α-amylase of green tea + brown rice showed a statistically significant difference among four treatment groups ($p < 0.01$).

Regarding to α-amylase inhibitory effects of six extracts, blact tea possessed relatively high inhibitory effect, as compared with acarbose as positive control coupound (Fig. 1). The values for green tea with 2.5 min. and 5.0 min were 27.6% and 33.5% on 1.5 g/50 ml, respectively. The values for green tea with 2.5 min. and 5.0 min were 36.8% and 41.1% on 1.5 g/100 ml, respectively (Fig. 2). The values for Oolong tea (wūlóngchá) with 2.5 min/50 ml and 5.0 min/50 ml were 39.5% and 64.3% on 1.5 g/100 ml, respectively. The values for black tea with 2.5 min/50 ml and 5.0 min/50 ml were 63.2% and 84.9% on 1.5 g/100 ml, respectively.

An IC_{50} value is the concentration of the extract required to inhibit the activity of the enzyme by 50% of the free radicals present in the system. Table 4 showed the IC_{50} values of the extracts with different extracte times. The IC_{50} for α-amylase of green tea ranged from 44.9 to 79.1 ug/ml. Among analyzed extracts, green tea + honey was the lowest α-amylase inhibition activety (IC_{50} was 404.5 ug/ml on 2.5 min. and IC_{50} was 204.0 ug/ml on 5.0 min.).

Table 2. The degree of inhibition (%) of α-amylase by green tea and/or additives at different extraction times and volumes

Type	Concentration (mg/ml)	Tim (minutes)		*t*-test
		2.5	5.0	
Green tea	1.5/50	14.17±2.20	17.22±2.93	0.863
	1.5/100.0	18.89±1.73	21.11±1.27	
Green tea + brown rice	1.5/50	22.22±2.55	35.01±2.50	13.035**
	1.5/100.0	20.56±2.68	33.89±2.10	
Green tea + lemon	1.5/50	20.83±1.44	23.89±0.96	2.314
	1.5/100.0	17.50±2.20	21.11±1.27	
Green tea + honey	1.5/50	2.50±1.67	6.39±2.41	0.324
	1.5/100.0	14.72±1.73	15.83±1.44	
Wūlóngchá	1.5/50	20.28±2.10	33.06±3.15	3.551
	1.5/100.0	14.44±2.10	18.33±2.20	
Black tea	1.5/50	32.50±1.44	46.67±1.44	0.837
	1.5/100.0	34.17±2.20	38.61±1.92	
F-test		1.235		
		1.038		

Data represented the mean ± SD from three replicates. ** = $p<0.01$.

α-glucosidase inhibitory effects

The results of the α-glucosidase inhibitory effects of six tea extracts in comparison with the standard (Acarbose) at 410 nm were shown in Table 3. It was observed that inhibition percentage values go on increasing with enhancements in concentration of research plant extracts in the assay mixture. α-glucosidase inhibition for green tea evaluated at 1.5 g/50 ml was 10.6% and that of 1.5 g/100 ml was 6.8% on 2.5 minutes. α-glucosidase inhibition for black tea evaluated at 1.5 g/50 ml was 33.9% on 5.0 minutes. The all values of α-glucosidase inhibitory for 5.0 minites of tea were higher than those of 2.5 minutes. The all groups (extract volumes and times) did not show a statistically significant difference ($p<0.05$).

Figure 3 was shown the rate of α-glucosidase inhibitory of Acarbose (positive control) and relative inhibitory rate for green tea extracts. The values for black tea with 2.5 min. and 5.0 min were 46.9% and 57.6% on 1.5 g/50 ml, respectively. The values for black tea with 2.5 min. and 5.0 min were 44.8% and 61.1% on 1.5 g/100 ml, respectively (Fig. 4).

The IC_{50} for α-glucosidase of green tea ranged from 117.9 to 125.9 ug/ml (Table 4). Among analyzed extracts, green tea + honey was the lowest α-glucosidase inhibition activety (IC_{50} was 667.6 ug/ml on 2.5 min. and IC_{50} was 334.2 ug/ml on 5.0 min.).

Table 3. The degree of inhibition (%) of α-glucosidase by green tea and/or additives at different extraction times and volumes

Type	Concentration (µg/ml)	Tim (minutes)		*t*-test
		2.5	5.0	
Green tea	1.5/50	10.57±1.76	13.63±1.37	1.405
	1.5/100.0	6.77±1.95	10.58±2.15	
Green tea + brown rice	1.5/50	17.24±2.52	22.61±2.59	2.392
	1.5/100.0	15.17±2.73	19.24±4.20	
Green tea + lemon	1.5/50	22.55±1.86	26.83±0.94	1.327
	1.5/100.0	17.96±1.87	22.27±0.89	
Green tea + honey	1.5/50	4.36±1.82	6.63±1.89	1.862
	1.5/100.0	2.01±0.78	5.04±1.38	
Wūlóngchá	1.5/50	21.83±4.12	26.82±2.93	3.551
	1.5/100.0	19.43±2.11	25.05±2.10	
Black tea	1.5/50	28.58±2.86	33.93±1.84	1.363
	1.5/100.0	20.83±2.76	28.44±2.58	
F-test		0.167		
		0.989		

Data represented the mean ± SD from three replicates. ** = $p<0.01$.

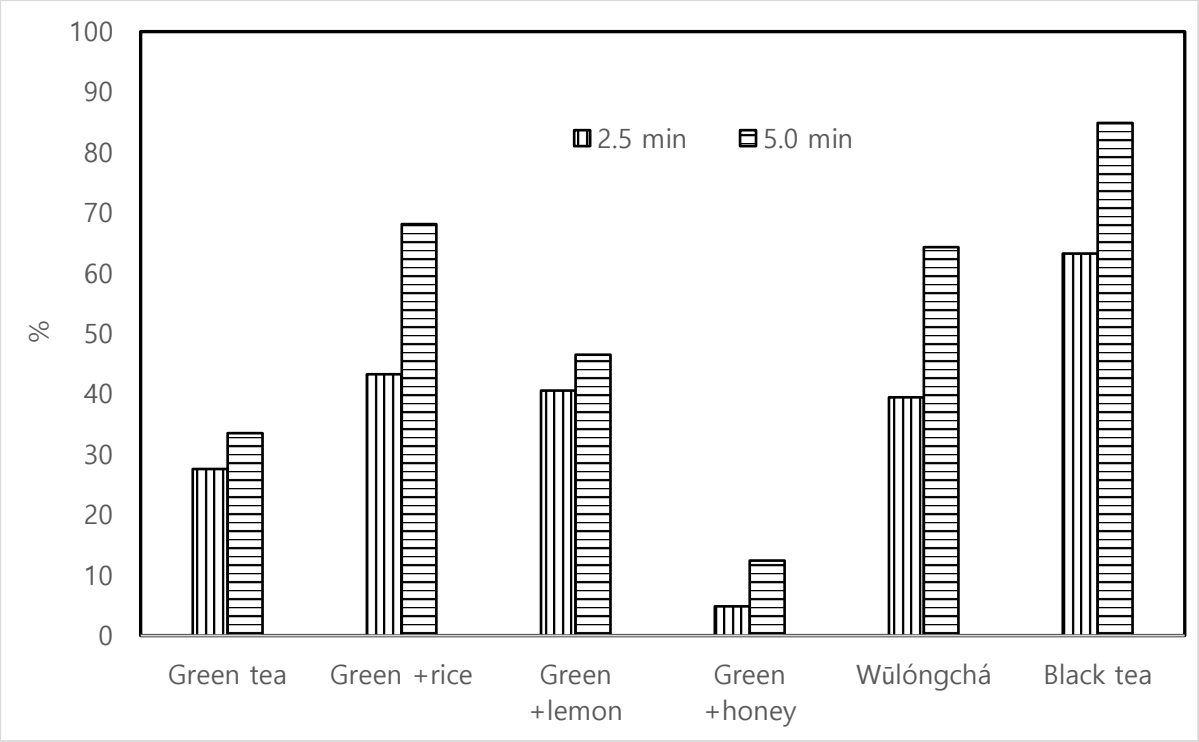

Figure 1. The rate of α-amylase inhibitory of Acarbose (positive control) and relative inhibitory rate for green tea and/or additives on 1.5 g/50 ml.

Figure 2. The rate of α-amylase inhibitory of Acarbose (positive control) and relative inhibitory rate for green tea and/or additives on 1.5 g/100 ml.

Table 4. The 50% inhibition (IC_{50}) of α-amylase and α-glucosidase of green tea and/or additives at different extraction times and volumes

Sample	α-amylase		α-glucosidase	
	2.5 min	5.0 min	2.5 min	5.0 min
Green tea	44.9	79.1	117.9	125.9
Green tea + brown rice	70.2	43.6	92.9	72.2
Green tea + lemon	78.9	69.3	75.0	63.9
Green tea + honey	404.5	204.0	667.6	334.2
Wūlóngchá	138.7	98.7	107.3	88.9
Black tea	78.0	61.1	108.6	87.6

Figure 3. The rate of α-glucosidase inhibitory of Acarbose (positive control) and relative inhibitory rate for green tea and/or additives on 1.5 g/50 ml.

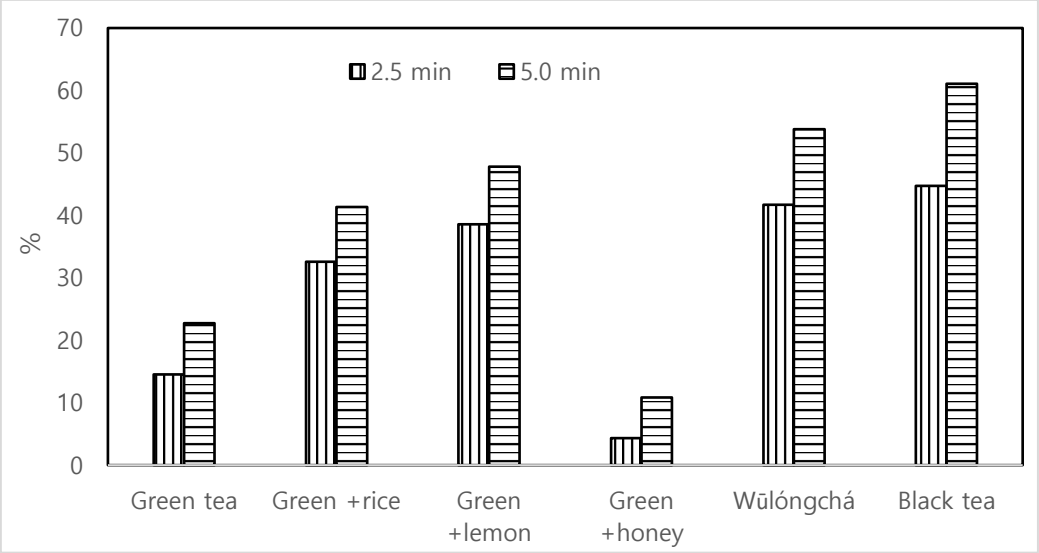

Figure 4. The rate of α-glucosidase inhibitory of Acarbose (positive control) and relative inhibitory rate for green tea and/or additives on 1.5 g/100 ml.

DISCUSSION

The effects of the food additives on the antibacterial activity of green tea extracts (GTE) suggested that the damage on cell surface is important in enhancing the antibacterial activity of GTE (Nakayama et al., 2008). In Korea, green tea with brown rice (hyeonmi-nokcha) is made by blending jeungje-cha (green tea that were steamed, not roasted, before being dried) leaves and roasted brown rice. Popular in both the loose and tea bagforms, brown rice green tea varieties are produced by Hankook Teaand Sulloc Tea (Jeong et al., 2012). 200 grams (7.1 oz) of brown rice green tea provides 610 kilocalories (2,600 kJ), 141.6 grams (4.99 oz) carbohydrate, 26 grams (0.92 oz) protein, 6.8 grams (0.24 oz) fat, and 12 milligrams (0.19 gr) sodium. The salivary amylase activity in the presence of fermented brown rice was significantly lower than that in the control, or for saliva in the presence of normal brown rice (Yamauchi, 2013). Lemon juice had a remarkable effect, completely interrupting gastric amylolysis by salivary amylase via a preliminary acidification of gastric contents (Freitas and Feunteun, 2019). These results provide a strong biochemical rationale for the development of dietary strategies to improve the glycaemic impact of starch-rich meals which could be tested in vivo. The standard in the tea industry is to sell tea bags or loose-leaf tea blends unsweetened, and allow users to add their own sweeteners. These can include sugar or honey.

The α-amylase present in honey hydrolyzed the starch chains to randomly produce dextrin and maltose. Honey heat treatment at 85°C reduced amylase activity 2 to 5 DN, but confirmed enzyme heat resistance (Babacan et al., 2006).

Green, oolong and black tea are all made from the same plant species, C. sinensis L. but differing in their appearance, organoleptic taste, chemical content as well as flavour due to their respective fermentation process (Sharangi, 2009)

The extent of starch hydrolysis by the tea leaves was as follows: green tea > oolong tea > black tea (Ismail et al., 2018). The low degree of hydrolysis for black tea was due to its strong inhibitory effect on α-amylase activity. Although, green, oolong and black tea leaves inhibit activity of α-amylase to different degrees due to their differing compositions, and structures of

phenolic compounds, the inhibits were shown in the order of black tea, oolong tea and green tea (Tables 2 and 3). Tea decoctions prepared from a number of black and green teas inhibited amylase in human saliva. Black teas gave higher levels of inhibition than green teas (Zhang and Kashket, 1998). Black tea decoction was significantly more effective than green tea, in agreement with the in vitro data.

Disclaimer FDA offers this list as a service to the Field Offices. Additives included are those specified in the regulations promulgated under the FD&C Act, under Sections 401 (Food Standards), and 409 (Food Additives). The Food Additives Status List includes short notations on use limitations for each additive. Although many additives are safe, some are not; natural food preservatives are generally considered to be the safest option for keeping foods fresh (Singh et al., 2012). For the morden food industry, natural food additives such as brown rice, lemon, and honey become important part of tea products.

Conclusion, black tea with food additives, oolong tea and green tea can be valuable in treatment of diabetes through inhibition of α-amylase and α-glucosidase.

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