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Relation of tea ingestion to salivary redox and flow rate in healthy subjects

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ABSTRACT

The biochemistry of human saliva can be altered by food intake. The benefits of tea drinking were extensively studied but the influence of tea ingestion on human saliva has not been revealed. The work aimed to investigate the immediate and delayed effect of vine tea, oolong tea and black tea intake on certain salivary biochemistry and flow rate. The saliva samples of healthy subjects were collected before, after and 30 min after tea ingestion. The chemical compositions and antioxidant capacity of tea samples were analyzed to correlate with salivary parameters. Principal component analysis indicated that the effects of vine tea consumption were dominated by increasing salivary flow rate (SFR), production rate of total protein (TPC), thiol (SH), malondialdehyde, catalase activity and antioxidant capacity (FRAP) in saliva. The antioxidant profile of studied tea samples (FRAP, polyphenols, flavonoids) was positively correlated with salivary SFR, TPC, SH and FRAP but negatively correlated with salivary uric acid concentration in saliva.

1. Introduction

Oral health is primarily maintained by saliva, a biological fluid secreted by salivary glands. It moistens and lubricates the oral surface, protects teeth against abrasion and attrition, prevents the dissolution of teeth and accelerates wound healing in the oral cavity [1]. The oral cavity is believed to be the first line of defencing against harmful substances in the human body, and the antioxidant status of saliva plays important role in such cases [2]. However, oral health is affected by some environmental factors such as food intake and cigarette smoking which can cause oxidative stress. The overproduction of free radicals (e.g. reactive oxygen/nitrogen species) leads to oxidative damage to cells and tissues in the oral cavity [2]. Saliva contains rich sources of antioxidants to keep redox balance in the oral cavity, which includes catalase, superoxide dismutase, transferrin, albumins and others. The sulfur-containing thiols also act as antioxidants in saliva [3]. The antioxidant system of saliva has been reported to be highly influenced by diseases such as diabetes, multiple sclerosis and early childhood caries [4-6]. As a biomarker of lipid oxidation, malondialdehyde (MDA) level is usually investigated for indication of oxidative stress in saliva. Salivary flow rate (SFR) has to be considered when studying saliva due to the variations that existed among individuals, ranging from approximately 0.3 mL/min to 0.4 mL/min and the composition of saliva is highly dependent on it [7].

Tea, generally made from Camellia sinensis, is popularly consumed around the world. It can be classified into green tea, oolong tea and black tea based on the level of fermentation, a process of converting catechins by enzymes. The polysaccharides, proteins, amino acids, polyphenols, organic acids, alkaloids and volatile compounds are the main chemical compositions in tea. As macronutrients, approximately 1.5% to 13% of polysaccharides and 15% to 23% of proteins were determined in tea depending on tea variety [8]. The main tea polyphenols include epicatechin, epigallocatechin, epicatechin-3-gallate and epigallocatechin-3-gallate.

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Due to the presence of polyphenol oxidases, catechins are oxidized to produce theaflavins, theaflagallins, theasinensins, theacitrins and thearubigens. Vine tea (VT) (*Ampelopsis grossedentata*) which belongs to Non-Camellia tea has been consumed for over a thousand years in China and Southeast Asia. High amounts of flavonoids, particularly dihydromyricetin (DMY), myricitrin and myricetin are present in VT. The major flavonoid, DMY can reach about 30% in dry leaves of VT [9]. The antioxidant activity of those polyphenols can be characterized by several assays such as FRAP, ORAC, DPPH and ABTS.

Although the antioxidant effects of tea have been extensively studied elsewhere, the influence of tea consumption on characteristics of saliva has not drawn much attention though it plays a significant role in oral health. The current work extended the previous study by including other 2 types of tea: vine tea and oolong tea (Tieguanyin). The healthy volunteers were studied as a group for analyses of saliva. Furthermore, certain tea compositions were investigated and antioxidant capacity of VT, oolong tea (Tieguanyin) and black tea (Lapsang souchong) were evaluated. The change of flow rate, protein, thiol and malondialdehyde content, uric acid concentration, α-amylase, catalase and antioxidant capacity of saliva by the consumption of tea were examined.

2. Materials and methods

2.1 Materials

The LS, TGY and VT were purchased from Wuyi Mountain Zhengshan Tea Industry Co., Ltd, China, Hangzhou Zhenyu Tea Co., Ltd, China and Guangdong Haoshuang Natural Health Care Food Co., Ltd, China, respectively. The 2,2′-Biquinoline-4,4′-dicarboxylic acid (BCA), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 3,5-dinitrosalicylic acid, acetic acid, ammonium molybdate, anthrone, concentrated sulfuric acid, Coomassie Brilliant Blue G-250, copper(II) sulfate pentahydrate, dihydromyricetin, disodium phosphate, ethanol, iron (II) sulfate heptahydrate, Folin-Ciocalteu reagent, gallic acid, glucose, glutamic acid, hydrochloric acid, hydrogen peroxide, iron (III) chloride hexahydrate, maltose, ninhydrin, phosphoric acid, potassium dihydrogen phosphate, sodium acetate, sodium bicarbonate, sodium carbonate, sodium chloride, sodium dihydrogen phosphate, sodium hydroxide, sodium tartrate, soluble starch, Tin (II) chloride and trichloroacetic acid (TCA) were obtained from Sinopharm Chemical Reagent Co., Ltd, Shanghai, China. The 1,1,3,3-tetraethoxypropane (TEP), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), bovine serum albumin (BSA), *L*-cysteine, thiobarbituric acid (TBA) and Tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma-Aldrich Co. Ltd, Shanghai, China. The potassium sodium tartrate was ordered from Guangdong Guanghua Sci-tech Co., Ltd, Guangdong, China. The uric acid assay kit was provided by Nanjing Jiancheng Bioengineering Institute, Jiangsu, China.

2.2 Participants

The research was conducted at Zhejiang Gongshang University, Hangzhou, China. 12 healthy volunteers (6 males and 6 females) aged between 22 and 31 years old attended the experiment. A consent form was signed by volunteers before saliva collection and details were described in previous work [10].

2.3 Tea brew preparation

For tea brewing, tea leaves (g) and boiling water (mL) were prepared at the ratio of 1:30. The same batch of tea leaves was brewed 2 times (15 s for each) and mixed well. The volunteers were asked to drink 200 mL of tea sample with a serving temperature of 50 °C. It should be noted the three tea samples were taken on separate days. For tea composition analyses, the same batch of tea brew (2 times) was mixed and centrifuged (500 × *g*) for 10 min at 25 °C using a centrifuge (T15A36, Hitachi Koki Co., Ltd, Japan) to remove impurities.

2.4 Saliva collection and salivary flow rate

According to previous studies with some modifications [10], saliva collection was conducted in the morning (9.30 AM–11.00 AM). The volunteers were asked to minimize the movement of face and lips with their head slightly tilted and the saliva was passively flowed from the bottom of oral cavity to a sterile centrifugal tube for 10 min, denoted as stage 1 (S1, baseline); after resting for 20 min, they were requested to consume and finish tea sample within 2 min and waited for 3 min before continuing another 10 min of saliva collection to minimize tea residues left in the mouth, denoted as stage 2 (S2, immediate effect by tea consumption). For stage 3 (S3), saliva was collected 30 min after tea consumption. Assuming 1 mL of saliva is equivalent to 1 g, SFR was calculated from the weight/volume (mL) of saliva divided by collection time (min). The collected saliva was instantly centrifuged (15 000 × *g*) at 4 °C for 30 min to remove the remaining residues and cell debris. The supernatant was transferred to centrifuge tubes after centrifugation and stored at −80 °C until analysed. The work was approved by the University Ethics Committee, School of Food Science and Biotechnology, Zhejiang Gongshang University with the reference number 20210707.

2.5 Analyses of tea brew

2.5.1 Total phenolic content

The total phenolic content (TPP) was determined according to the method proposed by Singleton and others with slight modification [11]. The diluted sample was mixed with Folin-Ciocalteu reagent and left at ambient temperature for 5 min. Na_2CO_3 was mixed with the solution and reacted for another hour. The absorbance was measured at 765 nm using UV-visible spectrophotometer (Hitachi's U-5100, Japan) and TPP was obtained from a standard curve of gallic acid.

2.5.2 Total flavonoid content

The total flavonoid content (TF) was determined by spectrophotometry against dihydromyricetin as standard. The method of Qin and others was adopted with slight modification [12]. 200 μL of the sample was mixed with 4.8 ml of 95% ethanol solution and

left at ambient temperature for 10 min. Absorbance was measured at 291 nm and concentration was expressed as DMY equivalents.

2.5.3 Polysaccharides

Anthrone-sulfuric acid assay was adopted for polysaccharides quantification. Diluted sample was added to 4 mL of anthrone-sulfuric acid solution in a boiling-water bath (Jinghong Experiment Equipment DK-S24, Shanghai) for 10 min and quickly cooled down to room temperature and absorbance was measured at 620 nm using glucose as standard.

2.5.4 Total protein content

Coomassie blue dye binding assay was applied for protein determination. 5 mL of Coomassie Brilliant Blue G-250 solution was mixed with 100 μL of sample and absorbance was read at 595 nm within 20 min. The total protein content (TPC) was estimated using BSA as standard.

2.5.5 Free amino acids

The free amino acids (FAA) were analysed by ninhydrin reaction. 400 μL of tea sample, 200 μL of PBS (67 mmol/L, pH 8.0) and 200 μL of ninhydrin solution were transferred to a test tube, followed by adding 1 600 μL of distilled water. The sealed test tube was placed in boiling-water bath for 10 min. 7.6 mL of distilled water was added to the mixture after it was immediately cooled down to room temperature. Absorbance was measured at 570 nm using glutamic acid as standard.

2.5.6 Antioxidant capacity

FRAP assay was employed to study the antioxidant activity. 20 μL of tea sample was transferred to 96-well microplate, followed by adding 150 μL of FRAP reagent which was prewarmed to 37 °C. The mixture was incubated at 37 °C in the dark for 10 min and absorbance at 593 nm was recorded using multi-mode microplate reader (Molecular Devices FlexStation 3, USA). The antioxidant activity was obtained from the standard curve of $FeSO_4 \cdot 7H_2O$.

2.6 Analyses of saliva

2.6.1 Total protein content

The TPC was measured using bicinchoninic acid (BCA) method. 100 μL of saliva was added to 200 μL of working solution containing BCA, sodium tartrate, $NaCO_3$, NaOH, $NaHCO_3$ and $CuSO_4 \cdot 5H_2O$ and incubated at 60 °C for 30 min. The mixture was transferred to 96-well microplate after cooling down to room temperature and absorbance was read at 562 nm. The concentration was estimated using BSA as standard.

2.6.2 α-amylase activity

The α-amylase activity (sAA) determination was described by Bernfeld [13]. 500 μL of 30 mmol/L soluble starch was added to 500 μL of diluted saliva in a water bath which was preheated to 37 °C for 3 min. 500 μL of color reagent solution was immediately transferred to the mixture and boiled for 15 min. 4.5 mL of distilled water was added to the resulting solution after it was cooled down to room temperature. The absorbance was read at 540 nm and sAA was calculated using maltose as standard.

2.6.3 Thiol content

According to the method reported by Taniguchi and others with slight modification, the saliva was diluted with 0.25 mmol/L Tri-HCl buffer solution (pH 8.3) in the ratio of 1 : 3. The solution was kept at room temperature for 3 h to allow maximum color development after adding 50 μL of 10 mmol/L DTNB, and absorbance was read at 412 nm. The thiol content (SH) was estimated using *L*-cysteine as standard [14].

2.6.4 Catalase activity

The catalase activity (CAT) measurement was proposed by Hadwan and Abed with slight modification [15]. 20 mmol/L of H_2O_2 solution was prepared and dissolved in 50 mmol/L of PBS (pH 7.4). The solution was diluted and standardized at an absorbance of 240 nm each time before use. The reagents were added and mixed well and absorption was read at 374 nm.

2.6.5 Uric acid concentration

The uric acid concentration (UA) was measured using uric assay kit based on manufacturer's instructions. After the reaction of saliva with reagents, the mixture was incubated for 10 min and absorbance was read at 510 nm.

2.6.6 Antioxidant capacity

The antioxidant power was estimated using FRAP assay which was previously explained.

2.6.7 Malondialdehyde content

The malondialdehyde determination (MDA) was described by Tarboush and others with some modifications [16]. 50 μL of saliva was mixed well with 200 μL of 1.04 mol/L TCA and 200 μL of 55.5 mmol/L TBA which was dissolved in 2 mg/mL of NaOH. The resulting solution was heated up at 100 °C for 15 min and cooled down to room temperature. The supernatant was taken after 10 min of 1 000 × *g* centrifugation. Absorbance was read at 532 nm and TEP was used as standard.

2.7 Statistical analysis

The statistical analysis of results was performed by XLSTAT 2019 (Addinsoft, USA). Significant differences ($P < 0.05$) for analyses of saliva among 3 stages and tea compositions in different tea samples were indicated by Analysis of Variance (ANOVA). The conversion

of concentration/activity of salivary parameters to production rate was calculated by multiplying SFR to study the correlations using Pearson correlation and Principal Component Analysis (PCA).

3. Results and discussions

3.1 Chemical compositions and antioxidant capacity of tea brew

The chemical compositions and antioxidant capacity of tea brew are tabulated in Table 1. The VT contained the highest amount of chemical compositions, followed by LS and TGY. ANOVA showed that TPP, TF, FRAP, TP, TPC and FAA was statistically and significantly different ($P < 0.05$) among the three tea samples. Low chemical compositions observed in TGY were possibly due to an insufficient brewing process. The tea leaves of TGY were curled during the curling process which limited the contact area between tea leaves and hot water in a short brewing time. In general, unfermented tea such as green tea contains higher polyphenols than fermented tea as polyphenol oxidase is deactivated at high temperature during the steaming process of green tea leaves. Our results revealed that slightly fermented VT contained higher TPP than TGY and LS. It was reported that about 420 mg GAE/g of TPP was extracted from 6 g of VT powder added to 300 mL of 70% ethanol solution for 30 min at room temperature [9], while our study showed a lower value of TPP in VT. This was probably because the concentration of chemical compositions of tea infusion was greatly dependent on the brewing method. Our experiment was designed to be consistent with the brewing method (e.g. brewing time) suitable for consumption in order to establish the correlation between chemical compositions of tea and biochemical compositions of saliva. Besides, a high TF of approximately 680 mg RE/g for VT extract was addressed by Jia's work, implying that the extraction method would be the main factor influencing the final content of VT extracts or infusions [9].

Our results were also in line with the work conducted by Nibir and others. The TPP and TF of unfermented green tea were about 4 times and 2 times higher than other fermented black tea varieties, respectively [17]. High TF in VT was attributed to the main flavanol compound, dihydromyricetin in tea leaves. About 170 mg/mL of dihydromyricetin was obtained in ethanol extraction at 25 °C and 20 mg/mL in water extraction at 80 °C [18]. For antioxidant capacity, a study found that green tea varieties performed significantly high FRAP than black tea varieties in Georgia [19]. Our work showed that the antioxidant capacity of VT was much higher than TGY and LS. The brewing time and temperature can greatly affect the antioxidant capacity of tea infusions. A 5-min brewing time for green tea exhibited the highest antioxidant capacity, and it was elevated while the brewing temperature was increased to enhance the solubility of catechins [20].

A high amount of TP, TPC and FAA was found in VT infusion, inferring that hot water could extract more of those compositions in VT than TGY and LS. There is limited literature available on tea proteins due to their insignificant role as a beverage [8]. However, there was more than 90% of water-insoluble proteins present in tea which could be extracted using the alkaline method, and high antioxidant activities were observed for those proteins from green tea, oolong tea and black tea [21]. The extraction of tea polysaccharides mainly uses hot alkali or hot water solution as the majority of bioactive polysaccharides are naturally polar. High level of TP was obtained from VT in the current study. The acid protein-bound heteropolysaccharides are water-soluble polysaccharides found in the leaves and stem from VT [22]. For free amino acids, VT contained a higher amount compared to another two tea infusions.

3.2 Salivary Flow Rate and Biochemistry

The highest SFR was 0.42 mL/min observed in S3 of VT, shown in Fig. 1a. The properties of food could influence the SFR. An increase of 25% fat content per dry matter would reduce about 19% of saliva incorporation [23]. It was reported that more saliva was produced for chewing tough meat rather than tender meat [24]. The effect of consuming liquid food such as tea infusions on saliva secretion would be little as high amount of saliva is not needed for bolus formation and swallowing. As shown in Fig. 1, the SFR was not significantly different ($P > 0.05$) among the stages for each particular

Table 1
The chemical compositions and antioxidant activity of Vine tea, Tieguanyin and Lapsang souchong.

Sample	TPP (μg GA/mL)	TF (μg DMY/mL)	TP (μg/mL)	TPC (μg/mL)	FAA (μg/mL)	FRAP (mmol Fe^{2+}/L)
Vine tea	765.0 ± 77.5^{a}	1 067.7 ± 26.0^{a}	631.9 ± 11.5^{a}	210.0 ± 9.4^{a}	59.7 ± 1.2^{a}	9.8 ± 0.5^{a}
Tieguanyin	41.9 ± 9.5^{c}	5.9 ± 1.1^{c}	86.3 ± 15.5^{c}	120.6 ± 10.9^{c}	23.2 ± 0.3^{c}	0.5 ± 0.1^{c}
Lapsang souchong	106.8 ± 14.1^{b}	44.0 ± 4.2^{b}	210.2 ± 11.9^{b}	146.3 ± 13.5^{b}	46.2 ± 0.3^{b}	1.3 ± 0.1^{b}

Note: Values displayed represent the means with 95% confidence interval. Means without the same letter indicate significant difference in particular parameter.

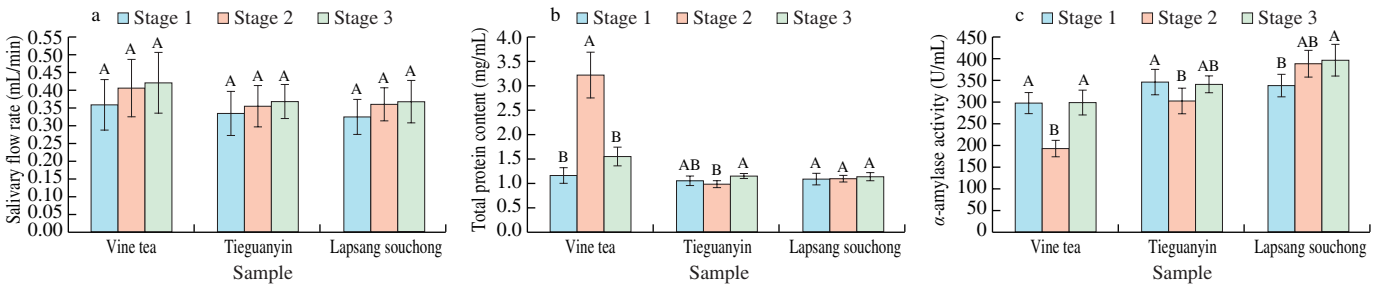

Fig. 1 The change of salivary (a) flow rate, (b) total protein content and (c) α-amylase activity after consuming different tea samples. Tea samples consumed before, after and 30 min are denoted as Stage 1, Stage 2 and Stage 3, respectively.

tea sample, indicating that tea consumption did not influence saliva secretion, although there was an obvious increasing trend of SFR found after tea drinking.

The consumption of three tea samples affected TPC of saliva differently (see Fig. 1b). The effect of tea consumption on the change of TPC of saliva is less reported. The range of TPC in the study was between 0.99 and 3.22 mg/mL. Studies showed that food consumption stimulated the secretion of several salivary proteins such as statherin, glycosylated and acidic proline-rich proteins [25]. The TPC was significantly increased ($P < 0.05$) after immediate VT consumption and returned to baseline 30 min later. For TGY consumption, insignificant decrease ($P < 0.05$) of TPC was observed in S2 and remarkable increase ($P < 0.05$) in S3, while drinking LS did not significantly ($P > 0.05$) influence TPC. The results suggested that the change of TPC of saliva was highly dependent on the type of tea consumed.

An average value of 326 U/mL for sAA was observed before tea consumption. After immediate drinking of VT and TGY, the sAA was sharply reduced ($P < 0.05$) but returned to baseline after 30 min (see Fig. 1c). A study showed that the inhibitory amylolytic activity of sAA was observed in green tea and black tea [26]. The inhibitory effect of α-amylase by dietary polyphenols was also reviewed [27]. The polyphenols, particularly flavonoids, are able to form hydrogen bonding with active site of α-amylase to suppress its activity [28]. High TF of VT greatly inhibited sAA in our study. In contrast, there was an increasing trend of sAA when LS was consumed. The results were in consensus with previous studies, stating that sAA was obviously elevated in half of the healthy subjects after black tea ingestion [10]. The results indicated that the consumption of VT and TGY exhibited an acute inhibitory effect on sAA but could be promoted by LS consumption.

As shown in Fig. 2a, immediate consumption of VT caused an insignificant increase ($P > 0.05$) of SH of saliva, followed by a significant decrease ($P < 0.05$) after 30 min (near to baseline), indicating that VT intake noticeably influences SH level. There was no significant difference ($P < 0.05$) found for SH as affected by TGY and LS consumption among stages. Several conditions could influence the antioxidant capacity of thiols in saliva. Salivary thiol level was reduced for Khat chewers compared with the control group [29].

A low level of salivary CAT was observed for Khat-chewers, smokeless tobacco users and smokers since the CAT was used to counteract and scavenge the overproduction of reactive oxygen species [29-31]. On the other hand, an increment of salivary CAT was reported for diabetic pregnant women and the reason could be explained as a trigger of antioxidant defence in saliva due to inflammation of chemokines, cytokines and cytokine receptors [32]. There was no significant change ($P > 0.05$) in CAT when VT and LS were consumed. TGY consumption had an effect on CAT, shown in Fig. 2b. A significant rise ($P < 0.05$) of CAT was found in S3 compared to S1 and S2, suggesting a progressive increase of CAT by TGY intake. We surmised that a higher level of certain compositions such as TPP, TP, TPC and FAA from VT and LS might restrict CAT production in saliva.

Approximately 70% of the total antioxidant activity of saliva is attributed by uric acid. An elevated level of UA was reported for patients with Down syndrome, severe early childhood caries and type 1 diabetes mellitus [6,33]. Smokers, smokeless tobacco users and patients with neurological disorders and tube-feeding caused a reduced UA [30,34-35]. However, the reasons for the change of UA due to those conditions were not clearly explained. The UA ranged from 20 μmol/L to 109 μmol/L in our study. The TGY and LS consumption did not significantly ($P > 0.05$) affect UA, as shown in Fig. 2c. In contrast, UA sharply and significantly decreased ($P < 0.05$) after immediate VT consumption and increased ($P < 0.05$) after 30 min. Studies showed that salivary uric acid was positively correlated with serum uric acid in young adults [36]. The cause of the sudden decrease of UA in saliva after VT intake was still unclear but we speculated that certain compositions in VT could suppress salivary UA.

A higher level of plasma FRAP was observed after green tea consumption when compared to the control treatment [37]. Another study showed that oolong tea ingestion increased plasma FRAP levels more significantly than placebo drinks [38]. However, the effect of TGY and LS consumption did not play a vital role in salivary FRAP

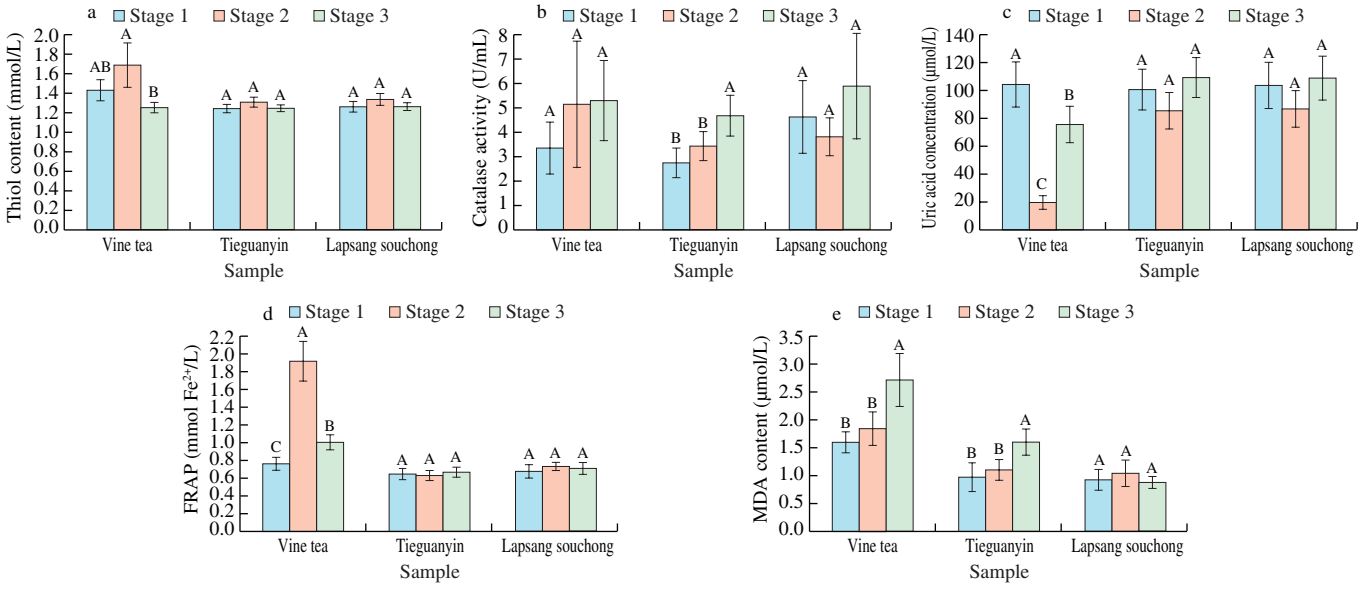

Fig. 2 The change of salivary (a) thiol, (b) catalase activity, (c) uric acid concentration, (d) FRAP and (e) MDA after consuming different tea samples. Tea samples consumed before, after and 30 min are denoted as Stage 1, Stage 2 and Stage 3, respectively.

Table 2
The average salivary flow rate, production rate of biochemical compositions and antioxidant capacity of human whole saliva before (Stage 1), after (Stage 2) and 30 min (Stage 3) after the consumption of different tea infusions.

Sample Unit	SFR (mL/min)	TPC (mg/min)	sAA (U/min)	SH (mmol/min)	CAT (U/min)	UA (μmol/min)	FRAP (mmol Fe^{2+}/min)	MDA (μmol/min)
				Stage 1				
VT	0.361	0.427	106.909	0.522	1.229	37.588	0.278	0.578
TGY	0.336	0.354	116.359	0.421	0.931	33.694	0.221	0.329
LS	0.325	0.358	109.169	0.469	1.512	33.633	0.223	0.303
				Stage 2				
VT	0.407	1.310	78.145	0.687	2.097	8.285	0.782	0.754
TGY	0.355	0.351	107.020	0.467	1.218	30.312	0.226	0.391
LS	0.361	0.398	139.295	0.610	1.381	31.235	0.266	0.375
				Stage 3				
VT	0.420	0.657	124.974	0.529	2.227	31.910	0.424	1.142
TGY	0.368	0.422	124.699	0.460	1.732	40.130	0.250	0.593
LS	0.367	0.417	145.116	0.462	2.166	39.962	0.263	0.323

in the current study. As shown in Fig. 2d, FRAP was not significantly influenced ($P > 0.05$) by TGY and LS consumption. The VT consumption performed significant effect ($P < 0.05$) on FRAP. The level was greatly improved from 0.8 mmol Fe^{2+}/L to 1.9 mmol Fe^{2+}/L after immediate VT consumption and reduced to 1.0 mmol Fe^{2+}/L 30 min later, indicating that drinking VT enhanced the antioxidant capacity of saliva for an extended period of time.

An elevated level of salivary MDA for smokeless tobacco users compared to the control group was reported [30]. A research work conducted by Zygula and others demonstrated that gestational diabetes mellitus patients who received insulin treatment had significantly higher salivary MDA than healthy pregnant women [39]. The change of salivary MDA concentrations by tea consumption is less discussed in the literature. The influence of LS consumption on salivary MDA was individually dependent but increased in the majority of previous studies [10]. Our results also showed an increase in MDA after LS consumption although significant changes were not found ($P > 0.05$). However, the VT and TGY ingestion affected salivary MDA, as explained in Fig. 2e. The MDA content in S2 was significantly increased ($P < 0.05$) compared to S3 for VT and TGY, revealing that drinking VT and TGY gradually raised the salivary MDA.

3.3 Correlations

The production rate revealed the amount of substance produced as a function of time. The concentration and activity of biochemical compositions of saliva were converted to production rate and tabulated in Table 2. PCA was applied to investigate correlations of a set of variables. Table 3 explains the factor loadings of analytical variables from F1 to F5 and biplot of the main principal components (F1 and F2) with observations were illustrated in Fig. 3. The large loadings were observed for SFR, TPC, SH, UA, FRAP and MDA that contributed to F1 while F2 was correlated with sAA and CAT.

The correlations among salivary parameters were studied by Pearson's correlation and correlation coefficients were shown in Table 4. The SFR was positively and significantly correlated with TPC, CAT, FRAP and MDA, while a strong positive correlation of TPC with SH and FRAP was also observed. Furthermore, a strong positive association between FRAP and SH was found. UA mostly and negatively correlated with the others, including TPC, SH and FRAP but positively correlated with sAA.

Table 3
Factor loadings of the analytical variables.

Variables	F1	F2	F3	F4	F5
SFR	0.826	0.510	0.109	−0.132	−0.106
TPC	0.973	−0.137	−0.058	0.127	−0.035
sAA	−0.580	0.678	−0.395	−0.163	−0.133
SH	0.828	−0.158	−0.361	−0.363	0.161
CAT	0.621	0.640	−0.222	0.375	0.100
UA	−0.847	0.470	0.111	−0.003	0.169
FRAP	0.983	−0.114	−0.058	0.087	−0.035
MDA	0.702	0.497	0.464	−0.183	0.036

Table 4
Correlation coefficient of the analytical variables of saliva.

Variables	SFR	TPC	sAA	SH	CAT	UA	FRAP	MDA
SFR	**1**	**0.713**	NS	NS	**0.752**	NS	**0.738**	**0.891**
TPC	**0.713**	**1**	NS	**0.795**	NS	**−0.887**	**0.998**	NS
sAA	NS	NS	**1**	NS	NS	**0.745**	NS	NS
SH	NS	**0.795**	NS	**1**	NS	**−0.789**	**0.814**	NS
CAT	**0.752**	NS	NS	NS	**1**	NS	NS	NS
UA	NS	**−0.887**	**0.745**	**−0.789**	NS	**1**	**−0.886**	NS
FRAP	**0.738**	**0.998**	NS	**0.814**	NS	**−0.886**	**1**	NS
MDA	**0.891**	NS	NS	NS	NS	NS	NS	**1**

Note: Values highlighted in bold indicate a significant level $\alpha = 0.05$, NS is denoted as not significant.

PCA was conducted to further investigate the relationship among salivary profiles influenced by tea consumption. The PCA biplot for relationships among the variables was demonstrated in Fig. 3. Component 1 and 2 were used to explain 85.82% of total variation of the data set. The variables (red lines) were distributed in three quadrants: SFR, MDA and CAT in quadrant 1; FRAP, TPC and SH in quadrant 2; UA and sAA in quadrant 4, and a strong positive correlation existed for these variables in a particular quadrant. FRAP, TPC and SH were negatively correlated with UA. There was no distinct or weak correlation found between CAT and SH. The distribution of observations (blue dots) was shown in PCA biplot. VT_S2 in quadrant 2 stood out with the highest FRAP, TPC and SH, suggesting that immediate VT consumption increased the production rate of FRAP, TPC and SH. As discussed earlier, uric acid is a major contributor to the antioxidant activity in saliva. A deeper study is required to understand the promoting effect of TPC and SH to FRAP

without the contribution of UA after immediate VT ingestion. High SFR, MDA and CAT were also found for VT_S3, indicating that SFR, the production rate of MDA and CAT was raised by VT consumption for an extended period of time.

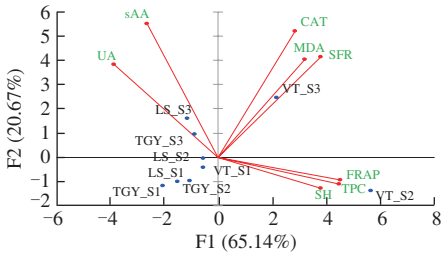

Fig. 3 Principal component analysis biplot for correlations among the salivary parameters. VT (Vine tea), TGY (Tieguanyin) and LS (Lapsang souchong) represents the tea variety and stage 1, 2 and 3 are expressed by S1, S2 and S3. Salivary flow rate, total protein content, salivary α-amylase activity, thiol content, catalase activity, uric acid concentration, ferric reducing antioxidant power (FRAP) and malondialdehyde content are denoted as SFR, TPC, sAA, SH, CAT, UA, FRAP and MDA, respectively.

Table 5
Correlation coefficient of the analytical variables between tea infusions and saliva in Stage 2 and Stage 3.

Variables	tTPP	tTF	tFRAP	tTP	tTPC	tFAA
			Stage 2			
sFR	**1.000**	**0.998**	**1.000**	NS	NS	NS
sTPC	**0.999**	**1.000**	**1.000**	NS	NS	NS
sAA	NS	NS	NS	NS	NS	NS
sSH	NS	NS	NS	NS	NS	**1.000**
sCAT	NS	NS	NS	**0.999**	NS	NS
sUA	NS	**−0.998**	NS	NS	NS	NS
sFRAP	**1.000**	**0.999**	**1.000**	NS	NS	NS
sMDA	NS	**0.998**	NS	NS	NS	NS
			Stage 3			
sFR	NS	**0.999**	NS	NS	NS	NS
sTPC	NS	**0.999**	NS	NS	NS	NS
sAA	NS	NS	NS	NS	NS	NS
sSH	**0.999**	**1.000**	**0.999**	NS	NS	NS
sCAT	NS	NS	NS	NS	NS	NS
sUA	**−0.998**	**−1.000**	**−0.998**	NS	NS	NS
sFRAP	**1.000**	NS	**1.000**	NS	NS	NS
sMDA	NS	NS	NS	NS	NS	NS

Note: Values highlighted in bold indicate a significant level $\alpha = 0.05$, NS is denoted as not significant. *t* and *s* are denoted as tea and saliva, respectively.

The chemical compositions and FRAP of tea samples were correlated with salivary profile after tea consumption and correlation coefficients were arranged in Table 5. An elevated level of TF in tea strongly increases TPC of saliva and SFR, while negatively influencing UA of saliva for immediate consumption of tea samples, and the effect lasted for 30 min. A positive correlation was found for TF of tea with FRAP and MDA of saliva in S2 and SH of saliva in S3. An animal study reported that tea polyphenols caused an increased level of rat serum CAT and a decrease in MDA [40,41]. However, TPP in tea did not influence CAT and MDA of saliva in our study. It was positively correlated with TPC, FRAP of saliva and SFR in S2. The positive effect was observed for TPP of tea with SH and FRAP of saliva but negatively affected UA of saliva 30 min after tea drinking. Furthermore, FRAP of tea would positively influence FRAP, TPC of saliva and SFR after the tea was just consumed. The observation of FRAP of tea was found similar to TPP of tea in S3. Results suggested that the antioxidant profile of tea (TPP, TF and FRAP) could elevate TPC, FRAP of saliva and SFR while directly consumed. Besides, SH and UA of saliva seemed to be positively and negatively correlated with the antioxidant profile of tea after 30 min tea consumption, respectively. Being as nutrients in tea infusions, TP, TPC and FAA did not significantly correlate with the properties of saliva in the study. However, TP and FAA of tea were positively correlated with CAT of saliva and SH of saliva in S2, respectively.

4. Conclusions

Overall, our work highlighted the influences of salivary biochemistry, redox status and flow rate by tea ingestion (VT, TGY, LS) in healthy adults. In tea brew analysis, VT contained the highest level of TPP, TF, TP, TPC and FAA, exhibiting greatest antioxidant capacity when compared with TGY and LS. VT consumption had dominant effect in salivary profile. TPC, MDA and FRAP of saliva was increased but reduction of sAA and UA was observed after VT ingestion. TGY consumption caused significant increase of CAT while LS ingestion stimulated sAA. PCA revealed that UA was negatively associated with FRAP, TPC and SH, and these three parameters were found to be positively correlated with each other. The acute VT ingestion raised FRAP, TPC and SH, and the increase of SFR, MDA and CAT were noticed 30 min after consumption. The correlation between tea and the production rate of saliva was analyzed. Results showed that the antioxidant profile of tea which included TPP, TF and FRAP would increase salivary TPC, FRAP and SFR when the tea was immediately consumed. The antioxidant profile of tea was positively correlated with SH but negatively with UA in saliva after 30 min. The current finding concluded that immediate tea ingestion, particularly VT, would influence the certain biochemical composition and redox status of saliva, and the effect could last for 30 min. In order to optimize the salivary biochemistry and redox status for improving oral health by tea ingestion, a wide range of tea types such as green tea, dark tea and other herbal teas should be included in future work. Furthermore, a greater number of volunteers is required to study the long-term effect of tea ingestion to establish a stronger connection between tea consumption and oral health after the acute and short-term effect is fully understood.

Conflict of interest

There are no conflicts of interest to declare.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.1016/j.fshw.2023.03.037.

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