The interindividual variation of salivary flow rate and biochemistry in healthy adults: Influence of black tea consumption

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ABSTRACT

Saliva is firstly interacted with food or drink during ingestion. Alteration of salivary biochemistry by black tea consumption is unclear and rarely discussed in literature. The current work highlighted the immediate and delayed effect of black tea drinking on the change of several salivary parameters, including flow rate, α-amylase and catalase activity, malondialdehyde, thiol, nitric oxide, hydrogen peroxide and total protein content. Twelve healthy subjects aged between 22 and 31 years old were recruited in the study. The saliva was collected before, after and 30 min after black tea consumption. The black tea intake generally caused an increase of salivary flow rate, total protein content, catalase, H_2O_2 and MDA but reduction in thiol and nitric oxide for majority of the subjects. Principal component analysis showed that the salivary flow rate, production rate of total protein content, nitric oxide, thiol and malondialdehyde of saliva were primarily significant for black tea consumption.

1. Introduction

Tea is one of the most widely consumed beverages in the world. A variety of tea such as green tea, white tea, yellow tea, oolong tea, black tea and dark tea is produced based on the level of fermentation level. About 78% of black tea is manufactured globally due to the high demand in Europe, America and Middle East (Sanlier, Gokcen, & Altug, 2018; Yuan, 2011). Theaflavin, thearubigin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate are the six common types of polyphenols presence in black tea (Lorenz, 2013). Tea drinking is believed to have various functionalities for human's health such as antioxidant, anticarcinogenic, anti-inflammatory and antimicrobial properties (Li & Zhu, 2016; Sanlier et al., 2018). Green tea and tea polyphenols could apparently protect against obesity, inflammation and fatty liver induced by high-fat diet, as well as regulate the intestinal flora disorder caused by *Salmonella typhimurium* infection in mouse models (Ma et al., 2020; Zhang et al., 2018; Zhang et al., 2020). Several studies have also reported that the tea polyphenols contribute health benefits to human (Afzal, Safer, & Menon, 2015; Zhang, Qi, & Mine, 2019). Furthermore, the catechins in green tea, thearubigins and theaflavins in black tea can inhibit the proliferation of cancer cell, regulate lipid and glucose metabolism and stimulate the immune function (Sanlier et al., 2018). A 4-week use of green tea extract significantly reduced the total blood cholesterol in postmenopausal women comparing to control group was reported (Dinh et al., 2019). Other than all those functional properties, the sensory properties of tea have also been extensively studied. A cup of good-quality tea brew delivers long lasting sweet taste sensation in the oral cavity as well as increases saliva secretion for a period of time (Chong et al., 2019).

In food oral processing, saliva is the first biological fluid that comes in contact with food when it is ingested before swallowing process. Whole saliva is a mixed fluid formed by parotid, submandibular, sublingual and other minor salivary glands in the oral cavity (Edger, 1992). A healthy person produces about 0.5–1 L of saliva in a day. The whole salivary flow rate can reach to 2.0 ml/min when it is stimulated by chewing (Watanabe & Dawes, 1988; Heintze, Birkhed, & Björn, 1983). The functions of saliva have been discussed, including the maintenance of moist condition to prevent abrasion in the oral cavity, dissolution and distribution of tastants around the location of taste buds, secretion of digestive enzyme, protection of oral mucosa, oesophagus and tooth (Dawes et al., 2015). Several salivary proteins and peptides such as amylase, mucin, statherin, histatin, lysozyme, lactoferrin,

lactoperoxidase and antibodies also exhibit antimicrobial activity (Pedersen & Belstrøm, 2019).

Other than salivary proteins, nitric oxide (NO), a compound present in saliva, can penetrate cell membrane to inhibit bacterial growth (Mobarak & Abdallah, 2011; Wink et al., 1991). Besides, the redox homeostasis in the oral cavity is maintained by various salivary antioxidant components which include peroxidase, catalase, superoxide dismutase, transferrin, albumins and others (Zukowski, Maciejczyk, & Waszkiel, 2018). A low capacity of antioxidant systems or elevated level of reactive oxygen species (ROS) can occur if the redox homeostasis is not achieved, causing the oxidative damage of lipids, proteins as well as nucleic acids (Knas, Maciejczyk, Waszkiel, & Zalewska, 2013). This oxidative stress can lead to the formation of malondialdehyde (MDA) from lipid peroxidation, and a high production of lipid peroxides will cause damage to cell integrity (Trachootham, Lu, Ogasawara, Nilsa, & Huang, 2008). Thiols are the organic sulfur-containing compounds found in saliva and act as antioxidants defencing against the oxidative stress and preventing tissue damage (Masoud et al., 2012).

The salivary biochemistry (e.g. enzyme activity, protein and antioxidant profile etc.) can be influenced by several conditions such as smoking, khat chewing, diseases (e.g. periodontal disease, diabetes) and orthodontic treatment (Astaneie et al., 2005; Buczko, Knas, Grycz, Szarmach, & Zalewska, 2017; Khalili & Biloklytska, 2008; Masoud et al., 2016; Motamayel, Falsafi, Goodarzi, & Poorolajal, 2017; Singh et al., 2018; Tarboush, Masoodi, Bdour, Sawair, & Hassona, 2019). The interaction of food with saliva in the oral cavity is complex, where sensory properties of food are perceived, the form of food is changed and a number of biochemical reactions occur. A few of studies discuss the tribology of food-saliva interaction to reveal the mechanisms of astringent and smooth sensation (Brossard, Cai, Osorio, Bordeu, & Chen, 2016; Chong et al., 2019; Rossetti, Bongaerts, Wantling, Stokes, & Williamson, 2009; Upadhyay & Chen, 2019). The effect of green tea consumption on salivary antioxidant status of smokers and the influence of holding or chewing green tea in the oral cavity on salivary hydrogen peroxide have been studied, however limited research focuses on the relationship among the physical properties and biochemical components of saliva as affected by the consumption of black tea (Azimi et al., 2017; Lambert, Kwon, Hong, & Yang, 2007).

The tea drinking undoubtedly provides various functionalities in human health. However, the effect of tea on the oral cavity is less drawn attention. The salivary biochemistry is necessary for investigation since the oral status can be attributed by saliva. The research aims to highlight the immediate and delayed effect of black tea consumption on salivary flow rate and certain chemical components (total protein content, α-amylase, catalase, hydrogen peroxide, thiol, MDA and NO) of human whole saliva and to establish the correlations among them. The results from the study would provide a basis of improving or altering the salivary biochemistry by black tea drinking.

2. Materials and methods

2.1. Materials

The black tea (*Lapsang souchong*) was purchased from Wuyi Mountain Zhengshan Tea Industry Co., Ltd, Fujian, China. The determination of salivary catalase, total protein content, hydrogen peroxide, malondialdehyde and nitric oxide assay kit were obtained from Beyotime Biotechnology, Shanghai, China. The assay kit of salivary α-amylase activity and thiol content were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China.

2.2. Participants

12 healthy subjects who aged from 22 to 31 years old were recruited in Zhejiang Gongshang University, Hangzhou, China. The criteria for participating the experiment included: (1) physically healthy; (2) not addicted to smoking and drinking alcohol; (3) no oral diseases and oral surgery in the past 3 months (such as wisdom teeth removal, orthodontic treatment, mouth ulcers, etc.); (4) no fever and flu; (5) maintain regular sleep and eating schedule. The participants were required to sign a consent form before participating the experiment of saliva collection.

2.3. Tea brew preparation

For tea brew preparation, the black tea leaf (g) and boiling water (ml) were prepared at a ratio of 1:30. The tea leaf was brewed for 2 times with brewing time of 15 s for each. The tea drinking volume and temperature were 200 ml and 50 °C, respectively.

2.4. Saliva collection & salivary flow rate

The saliva collection was divided into 3 stages based on the experimental design. In order to observe the continuous effect of black tea consumption on human whole saliva, the participants' saliva was collected before, right after and 30 min after tea drinking, denoting as stage 1, stage 2 and stage 3, respectively. The time of saliva collection was kept consistence at 10 am to avoid the influence of salivary flow rate by circadian rhythm. The participants were refrained from eating and drinking (except water) at least 1 h before the saliva collection. The mouth was rinsed with distilled water for 30 s to reach a neutral state. After 5 min adaptation to the environment they were asked to sit and relax with their head slightly bent down, avoid any movement of the lips and face. The saliva was then naturally and passively flowed from the bottom of the oral cavity to a 5 ml centrifugal tube for 5 min (stage 1). The participants were allowed to rest for 5 min before the second stage of saliva collection. During stage 2, the participants were told to drink 200 ml of black tea within 2 min. The saliva was expectorated and discarded for the first 30 s after consuming sample and started for a 5-minute saliva collection. The stage 3 was conducted 30 min after tea drinking and followed the procedure of stage 1. The saliva collection might need to be repeated on other day depending on whether the amount of saliva produced by the participants was sufficient for analysis. The collected saliva was immediately centrifuged ($15000 \times g$) for 30 min at 4 °C. The supernatants were then equally transfered to 1 ml centrifuge tubes and stored at -80 °C until analysed. Assuming 1 ml of saliva equals to 1 g, the salivary flow rate was calculated from the weight divided by the collection time and the unit was expressed as ml/min. The study was approved by University Ethics Committee, School of Food Science and Biotechnology, Zhejiang Gongshang University with a reference number of 20201208.

2.5. Assays

The bicinchoninic acid (BCA) method was adopted for the determination of total protein content in human saliva using bovine serum albumin (BSA) as standard (detection range 50 – 2000 μg/ml). For the activity of salivary α-amylase determination, iodine–starch colorimetric method was used. The α-amylase can hydrolyse starch to produce glucose, maltose and dextrin. According to the manufacturer's specifications, a blue complex is formed by the addition of iodine solution to the unhydrolyzed starch for a known concentration of the substrate and it was measured using UV–visible Spectrophotometer (Hitachi's U-5100, Japan). The hydrogen peroxide concentration of saliva was determined based on the theory of producing Fe^{3+} ions to Fe^{2+} ions oxidized by hydrogen peroxide, forming purple colour with xylenol orange in a specific solution. The catalase assay kit (minimum detection level: 1 U/ml) was used to detect the catalase activity of cells, tissues and other samples by colour reaction. Catalase catalyses hydrogen peroxide to form water and oxygen in the presence of sufficient hydrogen peroxide. The hydrogen peroxide residues are catalysed by peroxidase to oxidize chromogenic substrate and produce a red product called N-(4-antipyryl)-3-chloro-5-sulfonate-p-benzoquinonemonoimine with the

maximum absorbance of 520 nm. The catalase activity which catalyses hydrogen peroxide to water and oxygen per unit time and volume is obtained from the standard curve of hydrogen peroxide. The thiol content of saliva was estimated through the reaction of thiol compounds with 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) to produce a yellowish compound. Lipid peroxidation MDA assay kit was applied for the determination of malondialdehyde content in saliva. The reddish MDA-TBA adduct is formed by reacting MDA with thiobarbituric acid (TBA) in high temperature and acidic environment can be measured through colorimetry at the absorbance of 532 nm. The Griess reaction was used for the detection of salivary nitric oxide concentration. A nitrosating agent is produced by acidified NO_2^- reacts with sulfonic acid to generate the diazonium ion which is then coupled to N-(1-naphthyl) ethylenediamine. The formation of chromophoric azo-derivative is measured with the light absorption of 540 nm. The absorbance of the determination of total protein content, hydrogen peroxide, catalase, malondialdehyde, thiol and nitric oxide concentration was measured using Multi-Mode Microplate Reader (FlexStation 3, USA).

2.6. Statistical analysis

XLSTAT 2019 (Addinsoft, USA) was used to analyse the results. In order to investigate the effect of black tea consumption for the particular subject, Analysis of Variance (ANOVA) was performed to indicate the significant difference of the studied variables (salivary flow rate, total protein content, α-amylase activity, hydrogen peroxide concentration, catalase activity, thiol content, malondialdehyde concentration and nitric oxide concentration) among stage 1 (before tea consumption), stage 2 (immediately after tea consumption) and stage 3 (30 min after tea consumption). The results were converted to production rate by multiplying salivary flow rate for correlation analyses (PCA and Pearson correlation). The Principal Component Analysis (PCA) among the studied variables in difference stages was analysed. Pearson correlation was used to examine the correlations among the variables.

3. Results and discussion

3.1. Salivary flow rate and biochemical analyses of saliva

Fig. 3.1 illustrates the effect of black tea consumption on salivary flow rate of 12 healthy subjects. The salivary flow rate ranged between 0.08 (stage 1) and 0.538 (stage 2) ml/min. In general, the average value of unstimulated whole salivary flow rate is between 0.3 and 0.4 ml/min, while a lower mean value of 0.228 ml/min (stage 1) was obtained in our study (Pedersen & Belstrøm, 2019). It was seen that consuming black tea did not significantly ($p > 0.05$) influence the salivary flow rate of all the subjects except subject 1 whose flow rate of saliva was significantly increased ($p < 0.05$) after the black tea consumption. However, an increment of salivary flow rate was observed after drinking black tea for the majority of the subjects and the highest increase of flow rate (108%) was found in subject 1 compared to stage 1 (before tea drinking). Considering a short resident time in the mouth for tea drinking, the stimulation of salivary flow rate by a continuous chewing 1 g of paraffin wax for 10 min was increased to 2.2 ml/min for males and 1.5 ml/min in females, implying that mechanical chewing has dominant effect on salivary flow rate than drinking liquid food (Ono et al., 2007). When consuming the acid beverages such as ice tea, the salivary flow rate can be increased about 38% (~1.2 g/min) compared to water (~0.74 g/min) (Davies, Wantling, & Stokes, 2009). A high value of 95% confidence interval present in Fig. 3.1 revealed that the saliva collected on different days could cause the high variation of salivary flow rate for the studied subjects. Furthermore, the salivary flow rate can vary highly among individuals as it is dependent on several factors such as the size of salivary glands, gender, collection time of the day and the emotional state of the individual (Bolwig & Rafaelsen, 1972; Ericson, 1971; Heintze et al., 1983).

There are more than 2000 types of biologically functional proteins and peptides (e.g. mucins, cystatins, proline-rich proteins, α-amylases, α-defensins, statherin, histatins, etc.) present in human saliva, and the type and amount are dependent on individual's diet, gender, age,
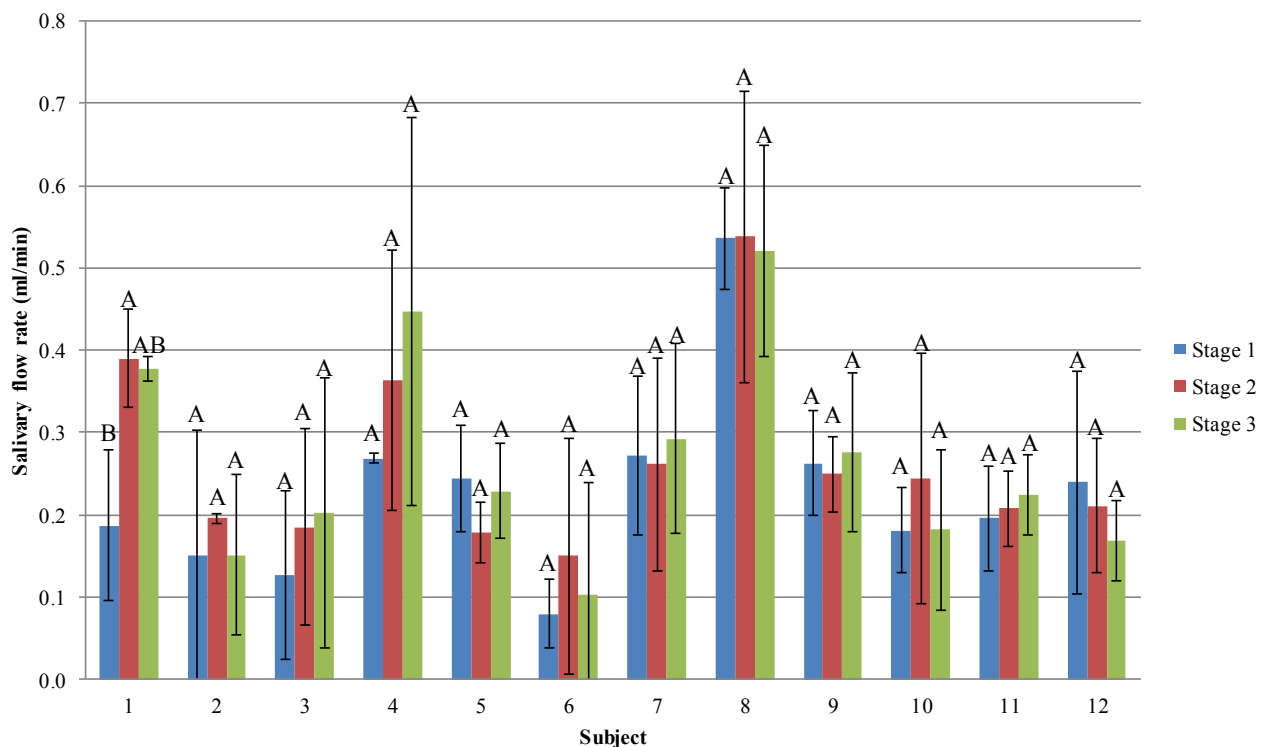

Fig. 3.1. The change of salivary flow rate as influenced by the consumption of black tea of 12 studied subjects. Stage 1: Before black tea consumption; Stage 2: After black tea consumption; Stage 3: 30 min after black tea consumption. Means without the same letter indicate significant difference in particular subject.

circadian rhythm and physiological status (Battino, Ferreiro, Gallardo, Newman, & Bullon, 2002; Khurshid et al., 2016). The influence of black tea consumption on total protein content of saliva is presented in Fig. 3.2. Aps and Martens (2005) suggested a total protein value of 3 mg/ml for whole human resting oral fluid, while other studies reported lower value of 2.6 mg/ml (Tarboush et al., 2019) and 2.16 mg/ml (De Sousa et al., 2015) for healthy individual. The average value of unstimulated saliva was lower than the reported value (1.2 mg/ml) in our study, indicating that the total protein content of saliva varies among individuals. Majority of the subjects produced higher protein content of saliva after tea drinking (stage 1 vs 2 or 3). The highest value (2.16 mg/ml) was found in stage 3 of Subject 2 whose initial protein content of saliva was also produced the most among others. Food ingestion especially after fasting period would stimulate the secretion of salivary proteins controlled by autonomic nervous system (Brandao, Soares, Mateus, & de Freitas, 2014). Significant differences ($p < 0.05$) was observed for all the subjects except Subject 8, suggesting that the consumption of black tea could positively affect the total protein content of saliva. The binding of black tea compounds such as tannins with salivary proline-rich proteins (PRP) could occur in the oral cavity while drinking. The structure of tannins (epigallocatechin gallate, (epicatechin-4β-8)-epicatechin and epicatechin-(4β-8)-epicatechin-3-O-gallate) were altered while binding to the same binding site of PRP (Canon et al., 2015). However, there was lack of information to explain the relationship between those bindings and the increase of salivary proteins after black tea drinking in the current study. Further analysis would be needed for studying the black tea compound-salivary protein interactions.

Salivary α-amylase, mainly found in parotid glands, involves in the cleavage of α-1,4-glycosidic bond from starch molecules to form maltose, dextrins and maltotriose. The enzyme exhibits antibacterial activity by providing nutrition for some bacteria through starch hydrolysis (Pedersen, Sorensen, Protor, Carpenter, & Ekstrom, 2018; Pedersen & Belstrøm, 2019). Fig. 3.3 demonstrates the salivary α-amylase activity as affected by black tea consumption. About half of the subjects' salivary α-amylase activity were significantly ($p < 0.05$) increased after or 30 min after consuming black tea, while 4 subjects were observed in the opposite way. Furthermore, drinking black tea did not significantly ($p > 0.05$) influence the salivary α-amylase activity of subject 1 and 9. A few of studies found that the binding of polyphenol with amylase caused the inhibition and conformational changes of the enzyme analysed by the combination of fluorescence quenching and differential scanning calorimetry (Sun, Wang, & Miao, 2020). The inhibitory effect of tea on amylolytic activity of saliva was also reported by Freitas and Feunteun (2019). At the neutral pH of the products, green tea and black tea performed 20–45% and 30–70% of inhibitory capacity on salivary α-amylase, concluding that the amylolytic performance of saliva is impaired by acid beverages comparing to pancreatin. The inhibitory effect of black tea on salivary α-amylase was not significant in our study was observed in subject 2, 4, 5 and 7. However, considering a 3-minutes incubation time of tea with saliva into 1:1 ratio, our study revealed an actual food-saliva system where a cup of tea was orally processed. The results suggested that the effect of black tea consumption on salivary α-amylase activity varied among the studied subjects and it positively influenced the activity in majority.

Hydrogen peroxide, a non-radical reactive oxygen species, is originally formed by bacterial metabolism in the oral cavity and also present in food such as coffee, honey and tea (Halliwell, Clement, & Long, 2000; Pedersen & Belstrøm, 2019). It is harmful to oral and gastrointestinal mucosa by oxidizing the detoxifying product of cyanide found in saliva called thiocyanate ion (Nagler, Klein, Zarzhevsky, Drigues, & Reznick, 2002). Fig. 3.4 shows the change of hydrogen peroxide concentration of saliva by the black tea consumption for the 12 studied subjects. It was seen that the concentration of hydrogen peroxide in saliva varied greatly among the individual, which ranged from approximately 2 to 223 μM in the study. As shown in the figure, consuming black tea played a
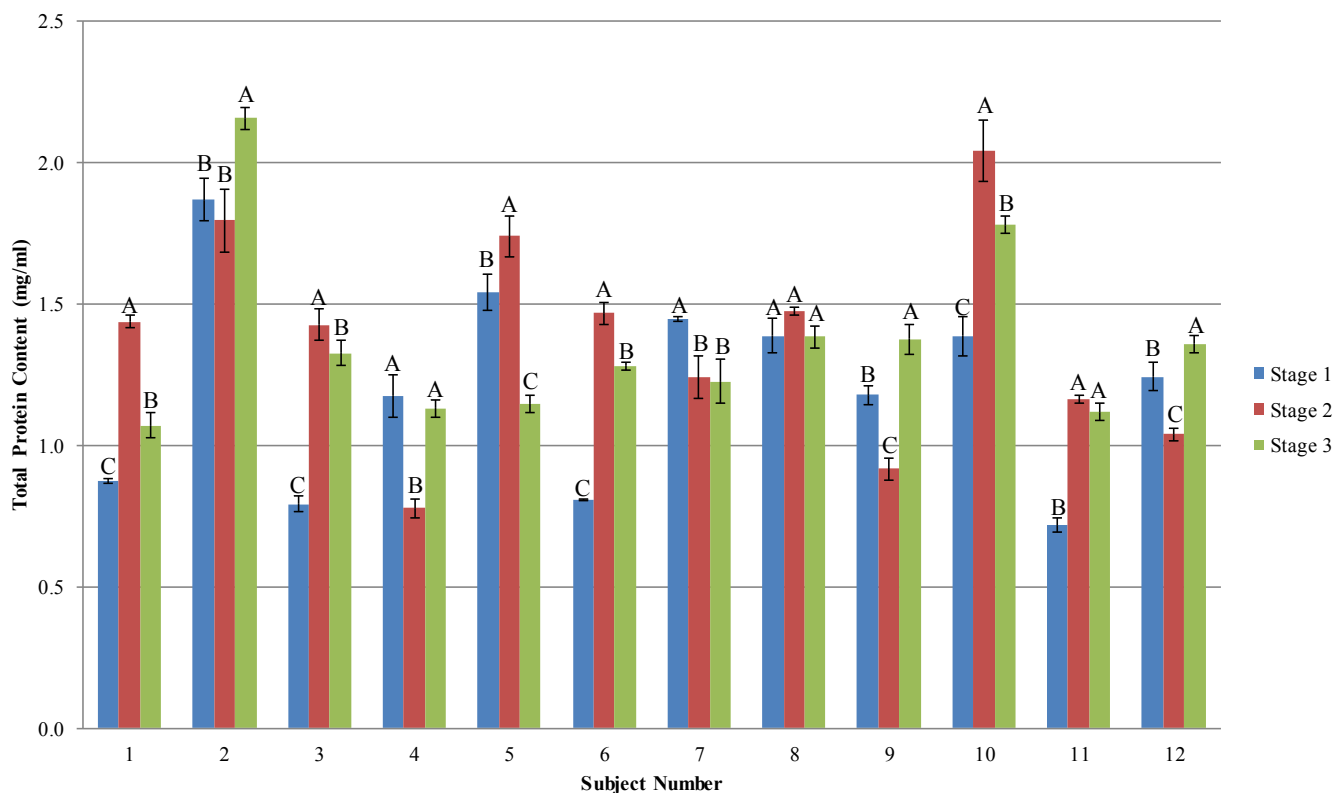

Fig. 3.2. The change of total protein content of saliva as influenced by the consumption of black tea of 12 studied subjects. Stage 1: Before black tea consumption; Stage 2: After black tea consumption; Stage 3: 30 min after black tea consumption. Means without the same letter indicate significant difference in particular subject.

Fig. 3.3. The change of salivary α-amylase activity as influenced by the consumption of black tea of 12 studied subjects. Stage 1: Before black tea consumption; Stage 2: After black tea consumption; Stage 3: 30 min after black tea consumption. Means without the same letter indicate significant difference in particular subject.

Fig. 3.4. The change of hydrogen peroxide concentration of saliva as influenced by the consumption of black tea of 12 studied subjects. Stage 1: Before black tea consumption; Stage 2: After black tea consumption; Stage 3: 30 min after black tea consumption. Means without the same letter indicate significant difference in particular subject.
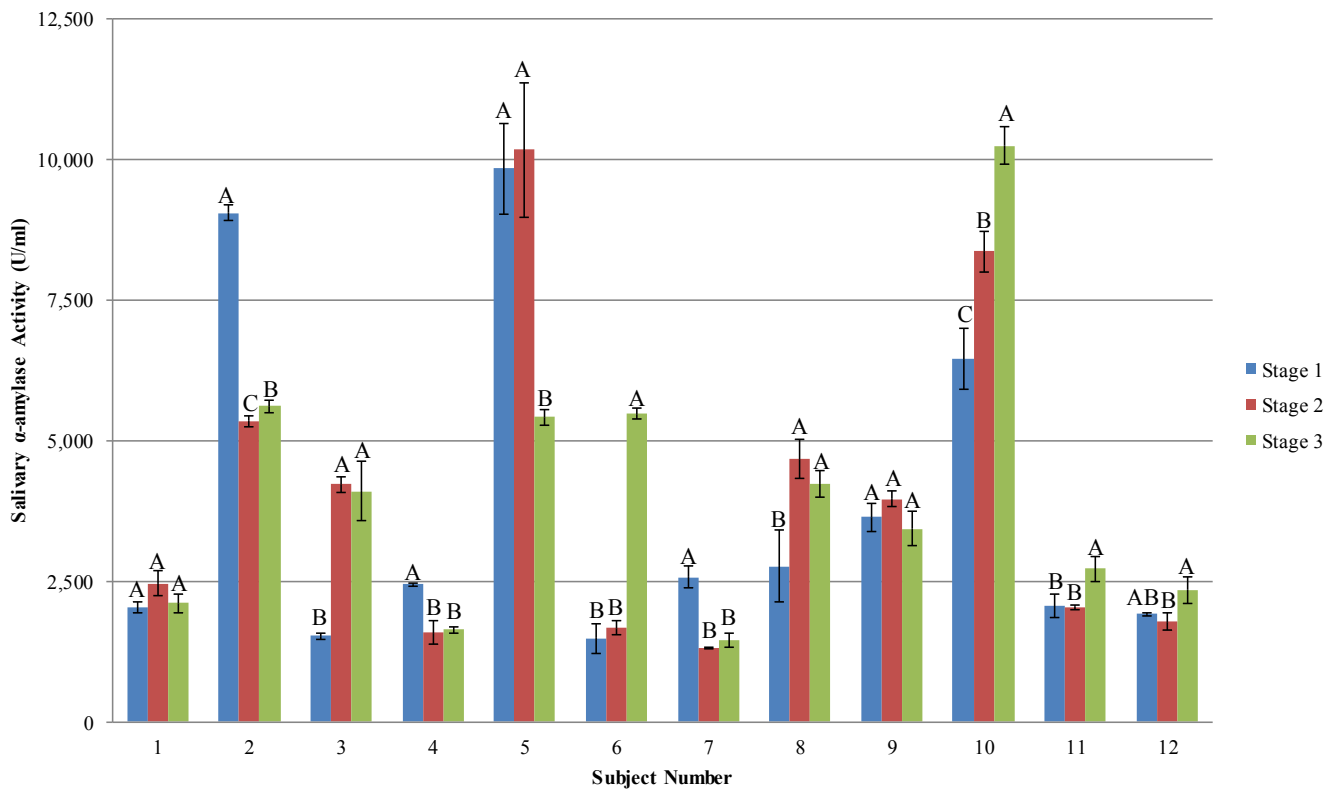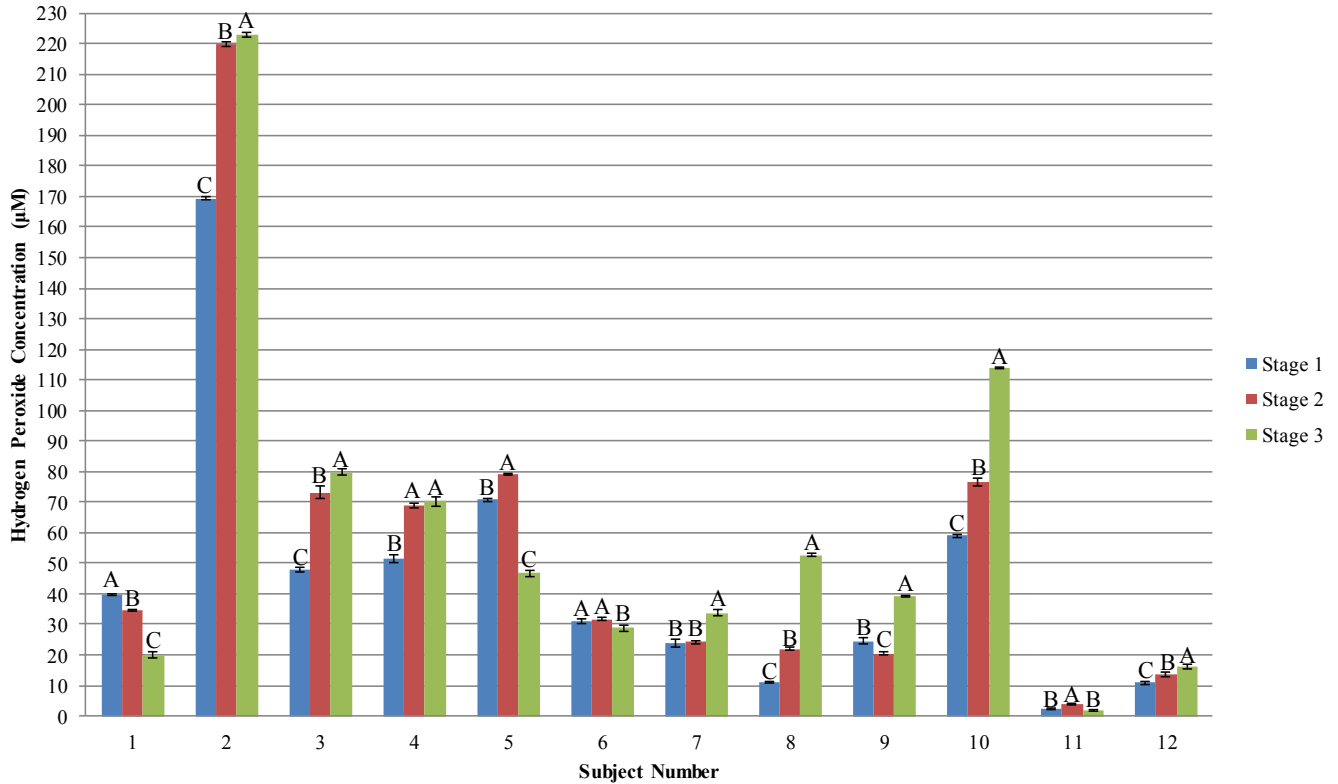
significant role for the change of hydrogen peroxide in saliva. 9 subjects' hydrogen peroxide concentration of saliva was significantly ($p < 0.05$) increased after black tea consumption (stage 1 vs 2 or 3) while the rest were in the opposite way. Drinking a warm tea creates a high oxygen partial pressure in the oral cavity, and the oxidative polymerization of the unstable catechins occur at the concentration of 2 to 160 μg/ml, to produce hydrogen peroxides (Lambert et al., 2007). This effect seemed to continue for 30 min after black tea consumption in about half of the studied subjects. Other studies showed that high concentration of hydrogen peroxide (above 88 μM) damaged tissue, exhibited carcinogenicity and promoted the formation of tumor with carcinogens (Naik, Tredwin, & Scully, 2006). However, keeping the concentration of hydrogen peroxide at 44 μM can maintain the oral health comparing to toothbrushing alone (Boyd, 1989). Based on our results, black tea drinking may not be recommended for those who contain high initial concentration of hydrogen peroxide in saliva such as Subject 2 and 10 (stage 1).

Catalase is one of the enzymatic antioxidants in the body and saliva. It catalyses hydrogen peroxide into water and oxygen (Chen & Scholl, 2005). Fig. 3.5 represents the effect of black tea consumption on salivary catalase activity of the subjects. The initial salivary catalase activity ranged from 9.1 to 577.8 U/ml was highly varied among the subjects. Several studies have been reported that cigarette smoke could inhibit or reduced the salivary catalase activity, which might be mainly due to the increase of synthesized free radical (Mendez-Alvarez, Soto-Otero, Sanchez-Sellero, & Lopez-Rivadulla Lamas, 1998; Motamayel et al., 2017; Singh et al., 2018). Furthermore, a significant decrease of salivary catalase activity was observed for khat chewers (Masoud et al., 2016; Tarboush et al., 2019). Amirmozafari, Pourghafar, and Sariri (2013) found that comparing to non-vegetarian group, a reduced salivary catalase activity was noticed for vegetarian group. The release of nickel or other metals from orthodontic treatment could also cause a reduction of salivary catalase activity by changing the function of bacteria and mucosa cells in the oral cavity (Buczko et al., 2017). In our studies,

majority (8 subjects) of the salivary catalase activity was increased significantly ($p < 0.05$) after or 30 min after the consumption of black tea, implying that black tea consumption has positive effect on salivary catalase activity. As a result, black tea drinking could improve the salivary defence system for people who has reduced level of salivary catalase activity.

The thiol content of saliva reveals the status of oxidative stress (Rho et al., 2005; Yoo et al., 2005). The change of salivary level of protein thiols by the consumption of black tea is illustrated in Fig. 3.6. An increase of salivary protein thiols was reported in obese patients compared to individuals with normal weight, and similar findings were also observed for benign and malignant brain tumour patients when comparing with healthy controls (Chielle & Casarin, 2017; Suma et al., 2010). The elevated level of salivary protein thiols in these patients was probably due to high demand of antioxidant systems against the oxidative damage (Lubrano & Balzan, 2015). On the other hand, crotonaldehyde and acrolein from cigarette smoke contains double bonds which can react with thiol groups to cause the functional and structural changes (Zukowski et al., 2018). The present study showed that the black tea consumption did not significantly ($p > 0.05$) influence the thiol content of saliva for 4 subjects (subject 8, 9, 11, 12) but significantly ($p < 0.05$) reduced right after drinking black tea for 7 subjects. The thiol in saliva seemed to be oxidized after black tea consumption (stage 2) and back to original level after 30 min (stage 3), indicating that black tea temporarily affected the thiol content of saliva. This could be due to the occurrence of oxidative stress (increase of ROS production) for oral tissues induced by elevated temperature during the ingestion of hot foods such as warm black tea (50 °C), causing an acute reduction of thiols in saliva (Zukowski et al., 2018).

Malondialdehyde, a product of unsaturated fatty acid peroxidation, is one of the indicators for oxidative stress (Wei, Zhang, Wang, & Chen, 2010). Certain medical conditions such as Down's syndrome and periodontal disease would lead to an increase of salivary MDA level than healthy individual (De Sousa et al., 2015; Khalili & Biloklytska, 2008).
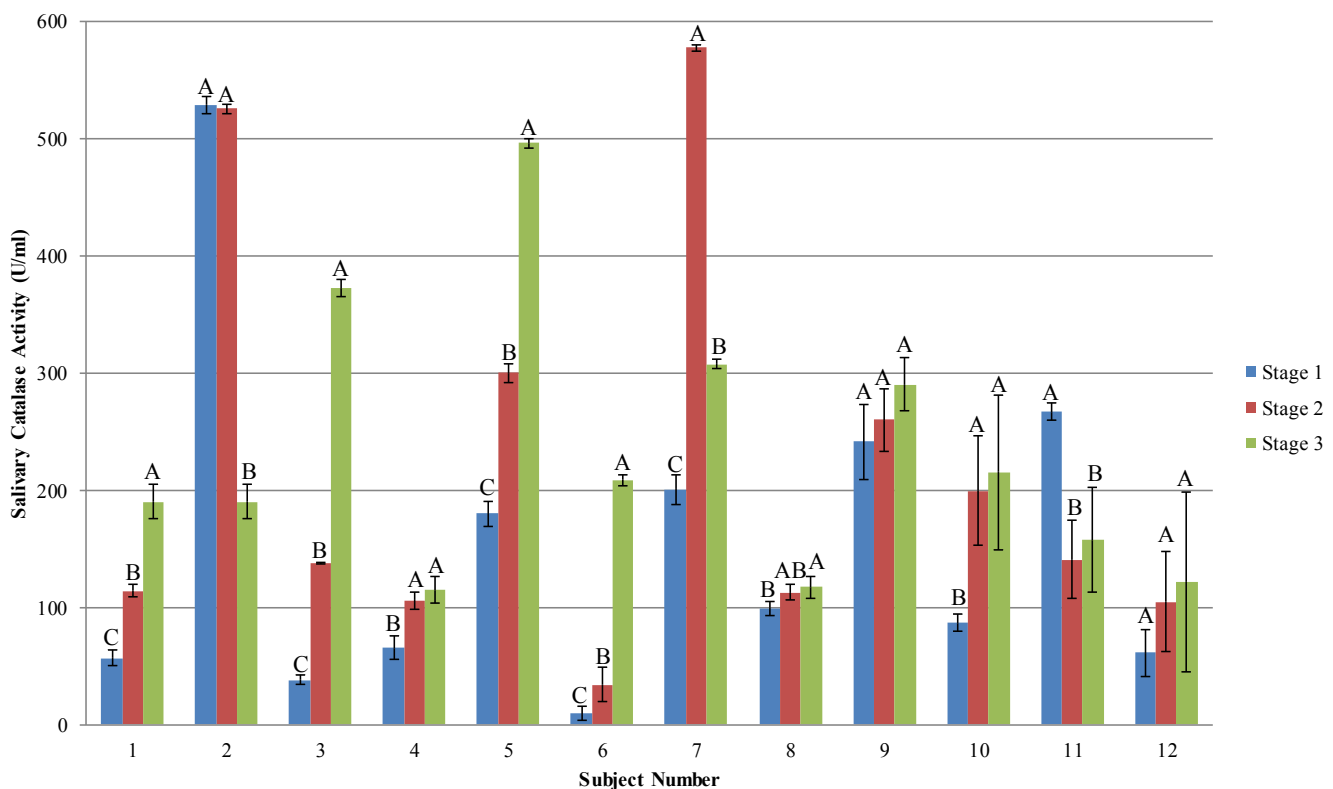

Fig. 3.5. The change of salivary catalase activity as influenced by the consumption of black tea of 12 studied subjects. Stage 1: Before black tea consumption; Stage 2: After black tea consumption; Stage 3: 30 min after black tea consumption. Means without the same letter indicate significant difference in particular subject.
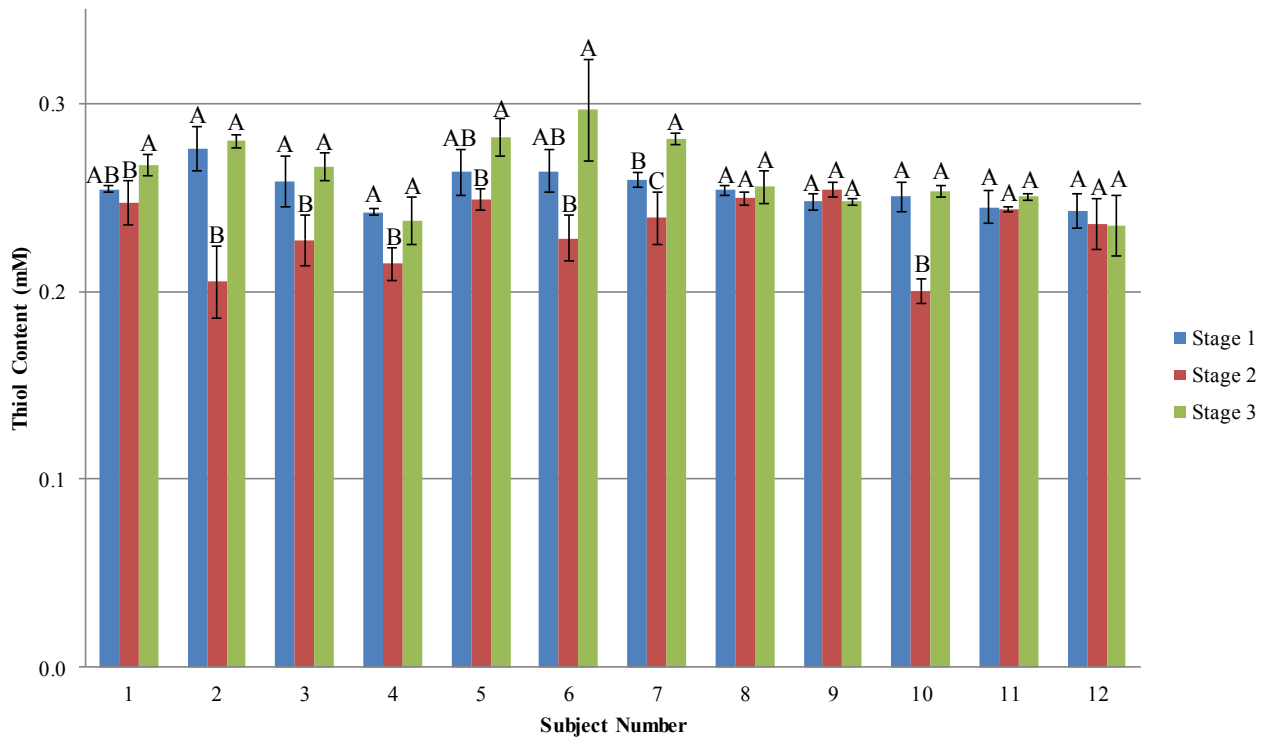
Fig. 3.6. The change of thiol content of saliva as influenced by the consumption of black tea of 12 studied subjects. Stage 1: Before black tea consumption; Stage 2: After black tea consumption; Stage 3: 30 min after black tea consumption. Means without the same letter indicate significant difference in particular subject.

Fig. 3.7. The change of malondialdehyde content of saliva as influenced by the consumption of black tea of 12 studied subjects. Stage 1: Before black tea consumption; Stage 2: After black tea consumption; Stage 3: 30 min after black tea consumption. Means without the same letter indicate significant difference in particular subject.
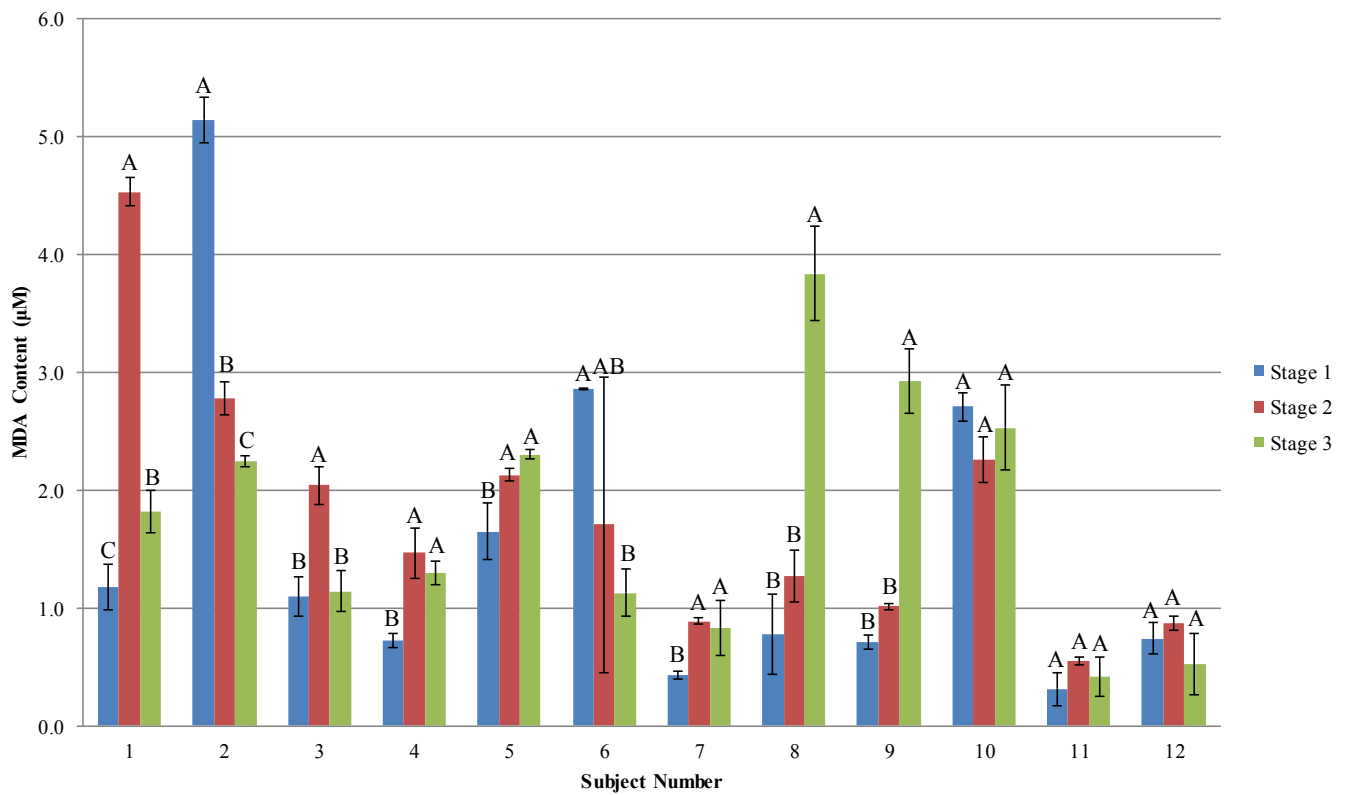
An elevated level of salivary MDA has been also positively linked with age, weight, khat-chewing, pregnancy and first week of orthodontic appliance attachment (Buczko et al., 2017; Chielle & Casarin, 2017; Gümüş, Emingil, Öztürk, Belibasakis, & Bostanci, 2015; Maciejczyk, Zalewska, & Ladny, 2019; Tarboush et al., 2019). The effect of black tea consumption on MDA content of saliva is presented in Fig. 3.7. There was no clear or distinct trend found and the effect varied among individual. However, a significant ($p < 0.05$) increase of MDA content was observed for 7 subjects after or 30 min after consuming black tea, while a significant ($p < 0.05$) decreasing trend was found in 2 subjects and no significant ($p > 0.05$) was found for the rest of the 3 subjects.

The reduction of nitrate forms nitrite which is further reduced to produce nitric oxide in saliva. The nitrate, comes from food intake as well as metabolism, is absorbed in the gut and delivered to blood and salivary glands through active transport (Bayindir, Polat, & Seven, 2005). Fig. 3.8 demonstrates the change of nitric oxide concentration of saliva as affected by the consumption of black tea, which ranged from 13 to 120 μM. A clear trend was shown in the figure that the nitric oxide concentration of saliva was significantly ($p < 0.05$) decreased after or 30 min after tea drinking for the majority of the subjects, while a significant ($p < 0.05$) increase was found for 4 subjects. A significantly high salivary nitric oxide level was reported in diabetic patients when comparing with healthy subjects (Astaneie et al., 2005). Besides, a poor oral hygiene such as dental plaque was associated with an increased level of salivary nitric oxide (Bayindir et al., 2005). Our results suggested that black tea drinking could inhibit or reduce salivary nitric oxide to a certain level for 8 subjects (subject 1, 3, 4, 5, 6, 9, 11 & 12).

3.2. Principal component analysis

The correlations of the physicochemical parameters of saliva from the 12 subjects were studied using principal component analysis. The production rate is expressed instead of concentration (unit mass per volume) to reveal the exact amount of the substance existed in the saliva per minute. The production rate of studied parameters of human whole saliva is tabulated in Table 3.1. Principal component analysis was used to analyse the results in Table 3.1 to study the correlation among these parameters.

The factor loadings of the analytical variables are presented in Table 3.2. The data indicates the variance explained by the variable on the particular factor (F). In general, F1 is most strongly correlated with TPC, followed by SH, SFR, AMY, CAT and NO while F2 is correlated with H_2O_2 and MDA in stage 1. For stage 2, F1 is highly correlated with TPC, SFR, SH, AMY, NO and MDA whereas H_2O_2 is correlated with F2. For stage 3, the F2 is correlated with CAT and F1 is correlated with the rest of the variables.

The Pearson's correlation coefficient, a measure of the strength of the association between two variables, is shown in Fig. 3.9. The black squares imply a high and statistical correlation between two variables. The positive and significant correlations ($p < 0.05$) were mainly observed in the study. It was clearly shown that a strong positive correlation found between SFR and SH, TPC and SH, SFR and TPC, while no significant correlation was observed for CAT and NO with others before black tea consumption (stage 1). The TPC was highly and positively correlated with SFR, AMY, SH, NO and MDA right after tea consumption (stage 2), suggesting that the concentration or activity of those variables was elevated when TPC was increased. Comparing to stage 1, the positive correlation of NO with TPC, AMY and H_2O_2 was greatly increased immediately after black tea consumption. For stage 3 (30 min after tea drinking), TPC was positively and significantly correlated with all the studied variables except CAT, and CAT did not correlate well with others in 3 stages. The correlation among these variables were further visualized by biplots under principal component analysis.

Fig. 3.10 illustrates the principal component analysis of the salivary flow rate and biochemistry of human whole saliva in 3 stages. The biplots consists of lines and dots to reflect variables and observations,
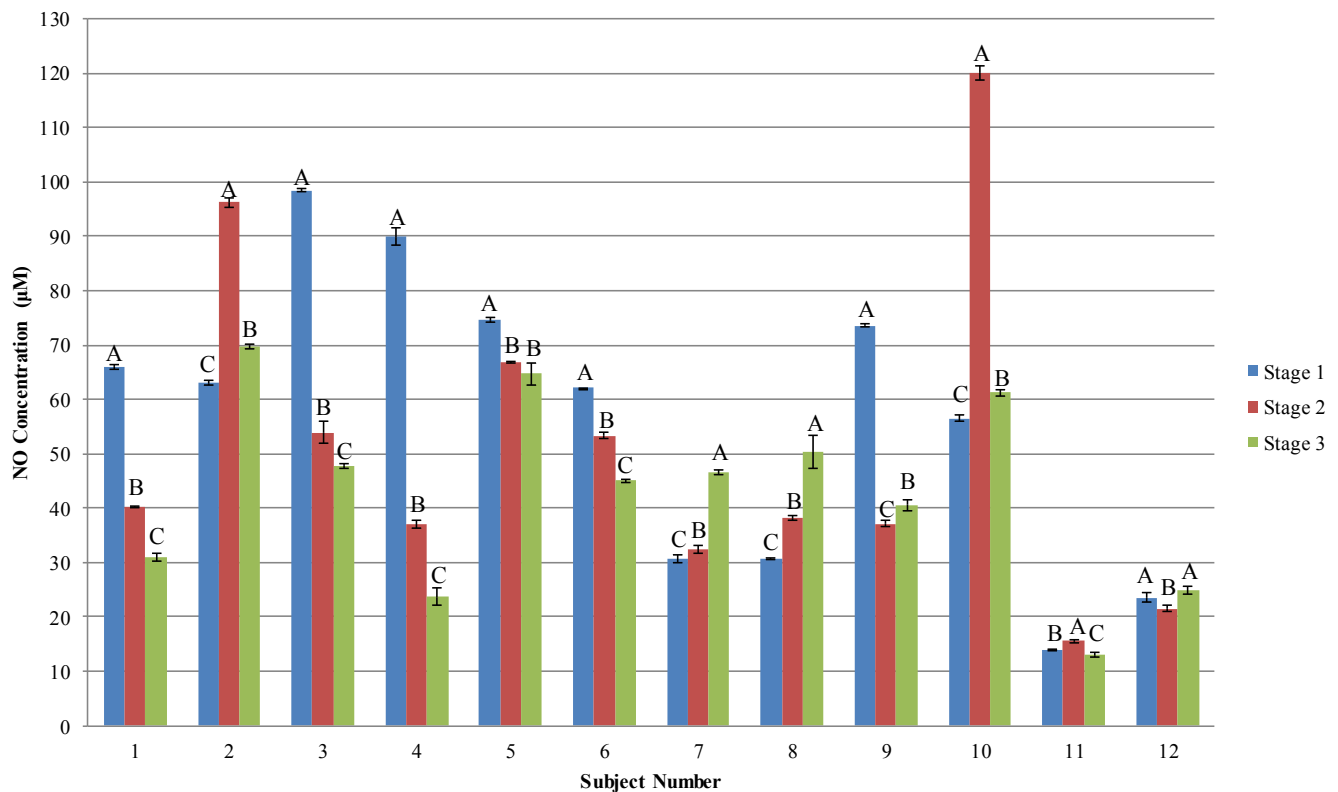

Fig. 3.8. The change of nitric oxide concentration of saliva as influenced by the consumption of black tea of 12 studied subjects. Stage 1: Before black tea consumption; Stage 2: After black tea consumption; Stage 3: 30 min after black tea consumption. Means without the same letter indicate significant difference in particular subject.

Table 3.1

The salivary flow rate and production rate of physico-chemical parameters of human whole saliva before (Stage 1), after (Stage 2) and 30 min (Stage 3) after the consumption of black tea.

Subject Unit	SFR ml/min	TPC mg/min	AMY U/min	H_2O_2 μmol/min	CAT U/min	SH mmol/min	MDA μmol/min	NO μmol/min
Stage 1								
1	0.187	0.163	379.343	7.423	10.588	0.048	0.220	12.340
2	0.151	0.281	1361.217	25.497	79.626	0.042	0.774	9.484
3	0.127	0.100	192.762	6.067	4.781	0.033	0.138	12.456
4	0.268	0.315	652.792	13.797	17.481	0.065	0.193	24.096
5	0.244	0.375	2396.584	17.266	43.832	0.064	0.402	18.173
6	0.080	0.064	117.701	2.464	0.727	0.021	0.228	4.940
7	0.272	0.393	698.572	6.496	54.433	0.070	0.116	8.334
8	0.536	0.744	1479.458	5.952	52.957	0.136	0.415	16.437
9	0.263	0.309	955.151	6.432	63.382	0.065	0.186	19.321
10	0.181	0.251	1166.766	10.674	15.709	0.045	0.490	10.235
11	0.196	0.140	404.885	0.458	52.222	0.048	0.061	2.742
12	0.239	0.297	456.273	2.615	14.568	0.058	0.177	5.631
Stage 2								
1	0.390	0.559	957.056	13.563	44.454	0.096	1.764	15.641
2	0.196	0.352	1047.061	43.142	103.000	0.040	0.545	18.859
3	0.185	0.264	781.645	13.545	25.438	0.042	0.378	9.973
4	0.363	0.282	573.885	24.945	38.328	0.078	0.532	13.440
5	0.178	0.309	1807.565	14.040	53.281	0.044	0.378	11.886
6	0.150	0.220	250.001	4.744	5.081	0.034	0.256	8.000
7	0.261	0.323	344.684	6.303	150.803	0.062	0.231	8.440
8	0.538	0.793	2509.113	11.829	60.387	0.134	0.681	20.536
9	0.249	0.228	987.260	5.059	64.796	0.063	0.252	9.256
10	0.244	0.499	2040.491	18.703	48.672	0.049	0.551	29.301
11	0.208	0.241	421.927	0.777	29.239	0.051	0.113	3.216
12	0.211	0.219	374.502	2.866	21.999	0.050	0.184	4.538
Stage 3								
1	0.378	0.404	796.797	7.525	71.761	0.101	0.687	11.685
2	0.151	0.326	846.598	33.674	28.704	0.042	0.339	10.532
3	0.202	0.268	827.162	16.112	75.239	0.054	0.230	9.647
4	0.447	0.505	731.136	31.386	51.348	0.106	0.578	10.595
5	0.228	0.262	1235.977	10.667	113.348	0.064	0.525	14.783
6	0.103	0.131	561.522	2.963	21.359	0.030	0.116	4.613
7	0.293	0.358	422.713	9.873	90.054	0.082	0.243	13.633
8	0.520	0.721	2201.988	27.350	61.004	0.133	1.996	26.217
9	0.276	0.380	947.999	10.870	80.079	0.068	0.807	11.198
10	0.182	0.323	1858.560	20.651	39.040	0.046	0.459	11.107
11	0.224	0.250	610.319	0.420	35.203	0.056	0.093	2.917
12	0.168	0.229	393.754	2.718	20.470	0.040	0.087	4.189

SFR = Salivary Flow Rate, TPC = Total Protein Content, AMY = α-amylase Activity, H_2O_2 = Hydrogen peroxide, CAT = Catalase, SH = Thiol, MDA = Malondialdehyde, NO = Nitric Oxide.

Table 3.2

Factor loadings of the analytical variables.

Variables	Stage 1		Stage 2		Stage 3	
	F1	F2	F1	F2	F1	F2
SFR	0.818	−0.563	0.859	−0.405	0.892	0.211
TPC	0.922	−0.325	0.960	−0.102	0.963	−0.084
AMY	0.814	0.369	0.770	0.191	0.717	−0.372
H_2O_2	0.481	0.826	0.358	0.803	0.597	−0.563
CAT	0.639	0.214	0.214	0.409	0.407	0.752
SH	0.840	−0.528	0.826	−0.493	0.904	0.267
MDA	0.512	0.735	0.685	−0.089	0.938	−0.089
NO	0.575	−0.013	0.769	0.510	0.931	0.033

SFR = Salivary Flow Rate, TPC = Total Protein Content, AMY = α-amylase, H_2O_2 = Hydrogen peroxide, CAT = Catalase, SH = Thiol, MDA = Malondialdehyde, NO = Nitric Oxide.

respectively. The number of the variables were reduced into two components which explained 77.80% (F1: 51.48%, F2: 26.33%), 71.27% (F1: 52.10%, F2: 19.17%) and 80.94% (F1: 66.53%, F2: 14.40%) of the total variation of the data set for stage 1, stage 2 and stage 3, respectively. It was clearly noted that the black tea consumption did not influence the correlation between the SFR and SH (positive correlation), revealing that high salivary flow rate would increase the thiol production in saliva with and without black tea intervention. Furthermore, the

correlation of SFR with H_2O_2 (no correlation) and TPC (positive correlation) was not affected among the 3 stages. It was also found that the SFR had positive effect on MDA and NO in stage 3 (see Fig. 3.9). Initially, the association between H_2O_2 and MDA was positively correlated, but turned into weaker after black tea drinking. Similar finding was observed for the correlation between AMY and CAT. Although CAT did not play a significant role among the studied variables, it was worth mentioning that the interaction between CAT and H_2O_2 became more positively correlated right after black tea consumption and negatively correlated after 30 min. While drinking black tea, the hydrogen peroxide was produced due to oxidative polymerization of unstable catechins in the oral cavity (Lambert et al., 2007). The elevated CAT was not sufficient to catalyse and compensate the excessive production of H_2O_2. However, after 30-minute black tea consumption, high CAT seemed to cause a lower production rate of H_2O_2 (see Fig. 3.10(c)), implying that the black tea consumption has a delayed effect on the production of CAT with a reduced H_2O_2 in saliva, or vice versa.

When observing the interaction of TPC with other variables, TPC initially had positive effect on AMY and SFR before drinking black tea. However right after the tea consumption, TPC was positively correlated with more variables such as MDA and NO, and H_2O_2 after 30 min. The finding suggested that an increase of TPC could elevate all the studied variables except CAT after 30-minute consumption of black tea. For the influence of black tea on SH of saliva, a positive correlation with TPC
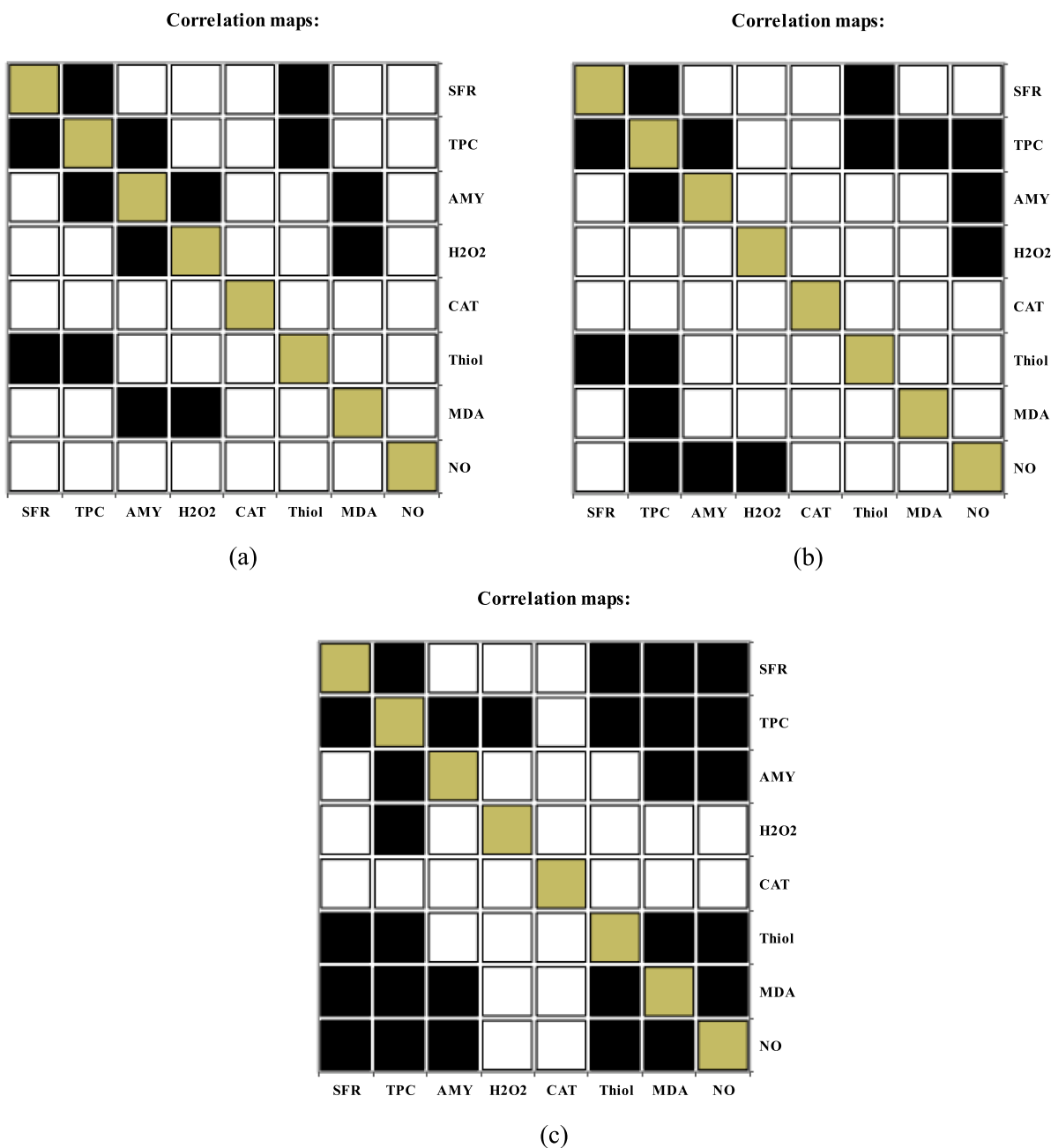
Correlation maps:

(a)

Correlation maps:

(b)

Correlation maps:

(c)

Fig. 3.9. Pearson's correlation of the analytical variables (a) before (Stage 1), (b) after (Stage 2) and (c) 30 min after (Stage 3) consuming black tea. The salivary flow rate, production rate of total protein content, α-amylase activity, hydrogen peroxide, catalase, thiol, malondialdehyde and nitric oxide are denoted as SFR, TPC, AMY, H_2O_2, CAT, SH, MDA and NO, respectively. The squares filled in black indicate significant correlations and white squares represent insignificant correlations.

and SFR was noticed in stage 1 and stage 2, while the MDA and NO were also found to be positively correlated with SH in stage 3. Furthermore, the NO content of saliva was not dominantly interacted with other variables without black tea drinking. The NO was more related to TPC, AMY and H_2O_2 in stage 2, and SH, MDA, TPC, AMY, SFR in stage 3.

The relationship between subjects and variables was illustrated in the biplots (Fig. 3.10). As shown in Fig. 3.10(a), the initial distribution of subject 2, 5 and 8 were unique among others. Subject 2 was related to H_2O_2 and MDA while subject 5 was related to CAT and AMY, and subject 8 was related to TPC, SH and SFR before black tea consumption (see Fig. 3.10(a)). When the black tea was just taken, a high CAT and H_2O_2 in saliva was found in subject 2, MDA in subject 1, SFR, TPC, SH and AMY in Subject 8. After 30-minute black tea consumption, subject 4 was associated with H_2O_2 whereas Subject 5 was associated with CAT, and subject 8 was closely related to TPC, MDA, SFR, SH, AMY and NO,

shown in Fig. 3.10(c). The results revealed that immediate drinking of black tea for subject 2 positively affected the CAT and H_2O_2, and MDA was reduced in saliva compared to stage 1 (see Table 3.1). Furthermore, CAT remained as an important variable for subject 5 in stage 1 and stage 3, while MDA had high influence to subject 1 in stage 2. The saliva of Subject 8 correlated most of the variables in the study.

4. Conclusions

The current study investigated the immediate and delayed effect of black tea consumption on the change of salivary flow rate and some salivary biochemistry to establish the correlations among them. The initial salivary concentration or activity (stage 1) of total protein content, α-amylase, hydrogen peroxide, catalase, malondialdehyde and nitric oxide greatly varied among the healthy individuals. The black tea
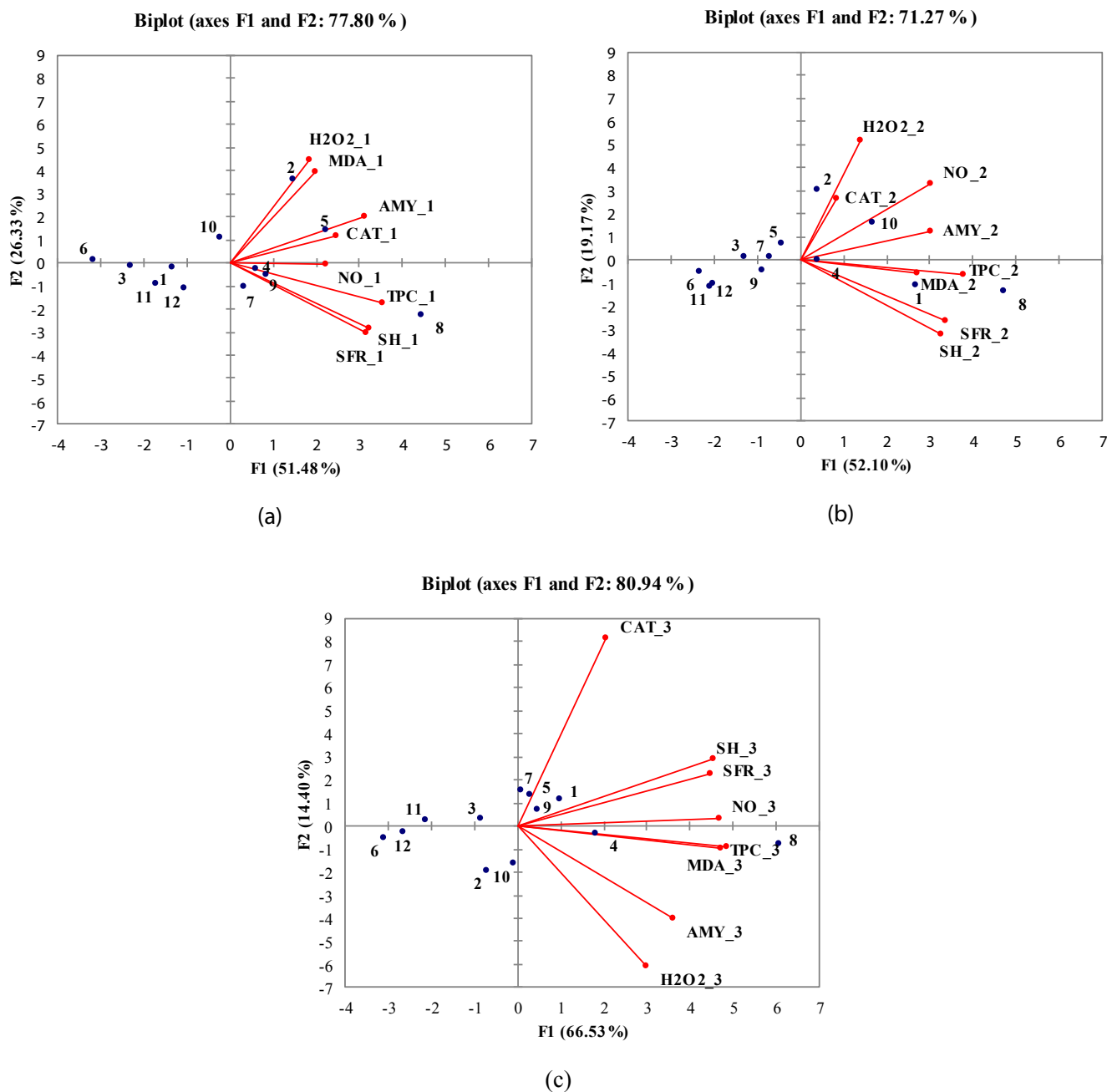
Biplot (axes F1 and F2: 77.80 %)

Biplot (axes F1 and F2: 71.27 %)

Biplot (axes F1 and F2: 80.94 %)

Fig. 3.10. Principal component analysis of the physico-chemical parameters of human whole saliva from 12 healthy subjects (a) before (Stage 1), (b) after (Stage 2) and (c) 30 min after (Stage 3) consuming black tea. The salivary flow rate, production rate of total protein content, α-amylase activity, hydrogen peroxide, catalase, thiol, malondialdehyde and nitric oxide are denoted as SFR, TPC, AMY, H_2O_2, CAT, SH, MDA and NO, respectively.

consumption led to an apparent increased concentration of salivary total protein content, α-amylase, hydrogen peroxide, catalase and malondialdehyde, and noticeable decreased concentration of thiol (stage 2) and nitric oxide for the majority of studied subjects. An immediate consumption of black tea significantly affected the total protein content, hydrogen peroxide and nitric oxide level of saliva in most cases. Furthermore, the delayed effect of black tea consumption was mainly observed in α-amylase and catalase activity, hydrogen peroxide, nitric oxide and total protein content in saliva. Correlation analysis showed a strong positive association among the SFR, production rate of TPC and SH in stage 1. The immediate (stage 2) and delayed effect (stage 3) of black tea consumption caused a positive correlation of TPC with the studied parameters except H_2O_2 and CAT in stage 2 and CAT in Stage 3. The black tea intervention did not affect the positive correlation SFR

with SH and TPC, concluding that the elevated salivary flow rate would increase the production rate of thiol and total protein in saliva. The results of the study suggested that the salivary flow rate and the studied biochemistry of human saliva were influenced by black tea consumption with the effect varied among healthy individuals. The production rate of TPC, MDA, NO, SH and SFR played significant role in black tea consumption.

Ethical statement

The human saliva collection study was approved by University Ethics Committee, School of Food Science and Biotechnology, Zhejiang Gongshang University with a reference number of 20201208.

CRediT authorship contribution statement

Pik Han Chong: Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Supervision. **Qiaojuan He:** Data curation, Investigation, Visualization. **Pingfan Rao:** Conceptualization, Validation, Supervision, Funding acquisition. **Li Li:** Supervision, Methodology. **Lijing Ke:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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