VIETNAM JOURNAL OF CHEMISTRY VOL. 53(2e) 162-166 APRIL 2015
DOI: 10.15625/0866-7144.2015-2e-038

CHANGES IN CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF FRESH OLD TEA LEAVES DURING STORAGE

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Received 23 January 2015; Accepted for Publication 18 March 2015

Abstract

Old tea leaves are a rich source of bioactive polyphenols. However unsuitable storage could cause the decrease in quality of this material. This work evaluated the change in EGCG, total polyphenol contents as well as the antioxidant, α-glucosidase and α-amylase inhibitory activities of the fresh old tea leave during 48 h under different storage conditions. The fresh old tea leaves were stored under 4 ^{o}C or at room condition. Total phenolic content, EGCG and caffeine in the tea leave extracts were quantified by HPLC analysis. α-glucosidase and α-amylase inhibitory activities of the extracted were evaluated by enzyme assays. The results showed that the EGCG, and total polyphenol contents in old tea leaves rapidly decreased after 48 h under room temperature condition while the sample storage at 4 ^{o}C retarded their decrease. The biological activity test showed that the inhibition of α-glucosidase and α-amylase decrease proportionally with the changes in EGCG, and total polyphenol contents. In contrast, the antioxidant of the old tea leaves during 48 h storage expressed by the DPPH scavenging capacity slightly augmented due to unknown mechanism. The storage of old tea leaves at room temperature caused the decomposition of EGCG and other polyphenols, which affected the quality of this material regarding its biological properties. The storage at 4 °C significantly protected samples from chemical decomposition and therefore conserved their quality.

Keywords. Epigallocatechin-3-gallate, green tea, total phenolic content.

1. INTRODUCTION

Green tea, made from the leaves and buds of *Camellia sinensis*, has been widely used around the world as a beverage and diet or healthful drink. The world green tea consumption has increased since there is scientific evidence for the long-purported health benefits of green tea. These effects are primarily based on the high content of catechins in green tea. Among these constituents, epigallocatechin-3-gallate (EGCG) is the most abundant antioxidant [1]. It has also proved that EGCG possesses a number of biological properties including anticancer, reducing risk of heart diseases, inflammation and diabetes [2-4]. In Vietnam, two kinds of green tea are mostly used: fresh and manufactured green teas. The later is generally produced from the tea buds and young leaves (1^{st}-3^{rd} leaves from the bud), however the fresh tea is prepared from the fresh old tea leaves (4^{th}-10^{th} leaves from the bud) by steeping them in boiling water for hours. This kind of tea is a favourite traditional beverage in Vietnam since it is believed to have counter-poison, lowering blood glucose, amelioration of obesity and health-enhancing abilities [5].

Although the fresh tea is widely used in Vietnam, the quality as well as the health effect of the drink made from of the old tea leaves have not been investigated. The old tea leaves are usually obtained after harvesting young leaves and buds. Then the materials were transported to the local market or stored in the polyethylene bags at room temperature for several days. Therefore, the quality of this material is not guaranteed and unable to be controlled. Furthermore, the storage of fresh tea leaves during several days in the market without suitable conditions can cause the decrease in quality of this merchandise since EGCG and other catechins in fresh tea leaves degrade due to the enzymatic oxidation. This raises the question about change in EGCG content as well as biological activities of the old tea leaves during storage. Therefore the current work aims to evaluate whether the changes in EGCG, caffeine and total polyphenol contents may cause the changes in antioxidant, α-glucosidase and

α-amylase inhibitory activities of old tea leaves during 48 h storage.

2. MATERIALS AND METHODS

2.1. Chemicals, reagents and apparatus

HPLC grade acetonitril, water and analytical grade formic acid were purchased from Merck. Epigallocatechin-3-gallate (> 97 %), caffeine (> 99 %), gallic acid (> 97 %), and reagents for bioassay were obtained from Sigma-Aldrich. The UV-Vis measurement was carried out by a JASCO V-630 spectrophotometer. Absorbance of the bioassay solution was read in a microplate reader (Molecular Devices, CA). The HPLC analysis was performed using an Agilent 1200 liquid chromatograph system equipped with a diode array detector

2.2. Green tea materials and sample extractions

The fresh old tea leaves (4-10th leaves from the bud) were collected in tea-growing areas in Vietnam: at Luong Son District (Hoa Binh Province) and at Dai Tu District (Thai Nguyen Province) in May 2010. A part of each sample was weighted (2 g), powdered and extracted with 10 mL water in a ultrasonic bath at 80 °C for 10 min. The extraction was repeated three times and the combined extracts were adjusted up to 50 mL in a volumetric flask. The remaining fresh leaves were stored in the fridge at 4 °C or kept in polyethylene bags at room temperature (RT). The similar extraction was repeated after 24 and 48 h for these samples. All extracts were filtered through a 0.45 μm filter unit before HPLC analysis and bioactivity tests.

2.3. HPLC analysis

HPLC separation was carried out in a Zorbax Eclipse XDB C_{18} column (4.6×150 mm) with a C_{18} guard column maintained at 35 °C. The elution was performed with a 20 min. gradient from 15 to 100 % acetonitril in water containing 0.1 % formic acid at flow rate of 0.4 mL/min. The injection volume was of 5 μL. The DAD acquisition wavelength for EGCG and caffeine detection was set at 280 nm. The standard stock solutions of EGCG and caffeine (1 mL/min) were accurately prepared in water and stored at 4 °C before used. The working solutions of the lower concentrations were prepared by the appropriated dilution of this stock solution. Before analysis, the working solution was filtered through a 0.45 μm filter unit.

2.4. Determination of total phenolic components

The total polyphenol content (TPC) was determined by Folin-Ciocalteu method according to the International Organization for Standardization (ISO) 14502-1 guideline [6]. The result was calculated based on the slope from serial dilution of a gallic acid standard. The final value was expressed as gallic acid equivalent (GAE).

2.5. DPPH radical scavenging activity

The antioxidant activity of the isolated compound was evaluated by its scavenging capacity of the DPPH radical. Briefly, tea leave extract (50 μL) was mixed with 150 μL of 150 μM DPPH solution dissolved in methanol. The plate was incubated in the dark at room temperature for 30 min. Then the absorbance of the reaction mixture was measured at 520 nm on a microplate reader.

2.6. Assay for α-glucosidase inhibition

The α-glucosidase (G0660, Sigma-Aldrich) enzyme inhibition assay was performed according to the previously described method [7]. Tea leave extract (50 μL) and 0.5 U/ml α-glucosidase (40 μL) were mixed in 70 μL of phosphate buffer (pH 7.0). After 5 min pre-incubation, 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution (40 μL) was added and the solution was incubated at 37 °C for 30 min. The absorbance of released *p*-nitrophenol was measured at 405 nm by using a microplate reader. The inhibitory activity was expressed as relative absorbance difference (%) of test sample to the absorbance change of the control where the test solution was replaced by water.

2.7. Assay for α-amylase inhibition

The α-amylase (A8220, Sigma-Aldrich) enzyme inhibitory activity was measured using the method reported by Kusano et al [8] with slight modifications. Substrate was prepared by boiling 100 mg potato starch in 5 ml phosphate buffer (pH 7.0) for 5 min, then cooling to room temperature. Tea leave extract (50 μL) and substrate (50 μL) were mixed in 30 μL of 0.1 M phosphate buffer (pH 7.0). After 5 min pre-incubation, 20 μL of 5 μg/mL α-amylase solution was added and the solution was incubated at 37 °C for 15 min. The reaction was stopped by adding 50 μL 1M HCl, and then 50 μL iodine solution was added. The absorbances were measured at 650 nm by a microplate reader.

3. RESULTS

3.1. Total polyphenol, EGCG and caffeine contents

The total polyphenol content (TPC) in the tea leaves during 48 hr storage were evaluated by Folin-Ciocalteu method and expressed as gallic acid equivalent. The result showed that the polyphenol constituents in the samples kept at room temperature significantly decreased. However, the degradation of polyphenol compounds could be delayed when the tea leaves were stored at 4 °C (table 1). It is reported that EGCG is a critical constituent for the green tea benefits [1, 9, 10]. Since the total phenolic constituents in fresh old tea leaves decreased during storage, we evaluated whether the EGCG level changes in the similar storage conditions. As showed in table 1, the EGCG content rapidly decreased by approximately 25% after 24 h and 55 % after 48 h under room temperature storage. In contrast, the EGCG level reduced approximately 30 % after 48 h keeping at 4 °C. Thus the storage at lower temperature (4 °C) could prevent the enzymatic processes in the fresh leaves and thereby reduce the degradation rate of EGCG. Caffeine is also known as a main constituent in green tea. However, the caffeine content decreased much slower than EGCG during 48 h storage under similar condition. This suggested that the decrease in TPC in the evaluated samples could be mainly due to the degradation of EGCG and related compounds.

Table 1: EGCG, caffeine and total phenolic content in the fresh old tea leaves during 48 hr storage

	Dai Tu sample			Luong Son sample		
Storage condition	EGCG (mg/g fresh)	Caffeine (mg/g fresh)	TPC (mg GAE/g fresh)	EGCG (mg/g fresh)	Caffeine (mg/g fresh)	TPC (mg GAE/g fresh)
0 h	28.2±2.5	39.2±3.4	282.4±12.5	31.7±3.6	45.7±7.3	296.6±16.4
24 h at 4 °C	25.7±3.8	38.3±4.0	275.1±15.1	29.5±3.1	44.0±4.6	281.2±13.7
24 h at RT	21.2±4.6	36.6±5.2	269.1±10.4	23.6±1.9	43.2±5.8	277.5±15.2
48 h at 4 °C	19.3±2.4	36.7±5.4	271.0±11.7	22.5±2.7	42.2±6.1	273.7±12.5
48 h at RT	12.6±1.9	35.5±3.7	232.3±12.9	14.7±1.1	41.1±3.5	242.4±18.1

3.2. DPPH scavenging, α-glucosidase and α-amylase inhibitory activities

It has showed that the total polyphenol content and the antioxidant activity are two parameters of quality for green tea. Since the TPC in the tea leaves decreased during storage, the antioxidant of the old tea leave extracts was then evaluated by the DPPH radical scavenging capacity. As shown in Fig. 1A, the antioxidant of the extracts slightly increased during storage. This observation was inconsistent with several reports which showed that the TPC determined by the Folin-Ciocalteu test are proportionally correlated with the antioxidant capacities [11, 12]. Thus the TPC might not be primary antioxidant constituents in the old tea leaves or unknown antioxidants could be produced during storage.

In contrast to the DPPH radical scavenging capacity, the inhibitory effects of the old tea leave extracts on the α-glucosidase and α-amylase were significantly decreased during 48 hr storage (Fig. 1B-C). Initially, tea samples showed 71-81 % inhibitions on both enzymes. At room temperature condition, the inhibitions dramatically decreased and reached only 49-56 % after 48 h. Similar to the results obtained from the chemical investigation, the α-glucosidase and α-amylase inhibitory activities of old tea leave stored under 4 °C slightly decreased in comparison to those kept at room temperature. It has been known that the inhibition of α-glucosidase and α-amylase, two enzymes responsible for carbohydrate digestion and glucose absorption, can lower hyperglycaemia and ameliorate diabetes [13, 14]. Previous studies have showed that catechins and other polyphenols in green tea are main inhibitors of α-glucosidase and α-amylase [15, 16]. Consistently, the present study indicated that the diminution in enzyme inhibitory effects occurred parallelly with the decrease in EGCG and TPC.

4. DISCUSSION AND CONCLUSION

Old tea leaves, which are not used in the production of green and fermented tea, are considered as agricultural waste. However, old tea leaves contain high phenolic content and possess potential biological activity. Therefore they could be used for supplement or preservation purposes in

food formulations [9, 17]. Several studies showed that the supplement of the old tea leave extract could enhance the stability of rapeseed oil and delay the oxidation of biscuit cream [18, 19]. For a long time, the Vietnamese people have their habit of drinking tea made from fresh old tea leaves. However, the health benefit of this drink is not guaranteed due to the uncontrolled quality of the materials. Our result showed that the quality of fresh tea leaves in term of polyphenol and EGCG contents together with the inhibition of α-glucosidase and α-amylase remarkably decreased after 24 and 48 h at room temperature. Normally, the fresh tea leaves stay in the market for several days under atmospheric condition. Thus the consumers may drink the low quality tea from the old tea leaves with decreased polyphenol and EGCG components. In fact, the quality and health benefits of uncontrolled fresh old tea leaves have not been evaluated in Vietnam. It is important to note that Vietnam is one of the biggest producers and exporters of green tea in the world [20], i.e. a big quantity of old tea leaves is available for exploitation. The result of the present study could be helpful to better use of old tea leaves, not only for the preparation of the fresh tea but also for other applications.

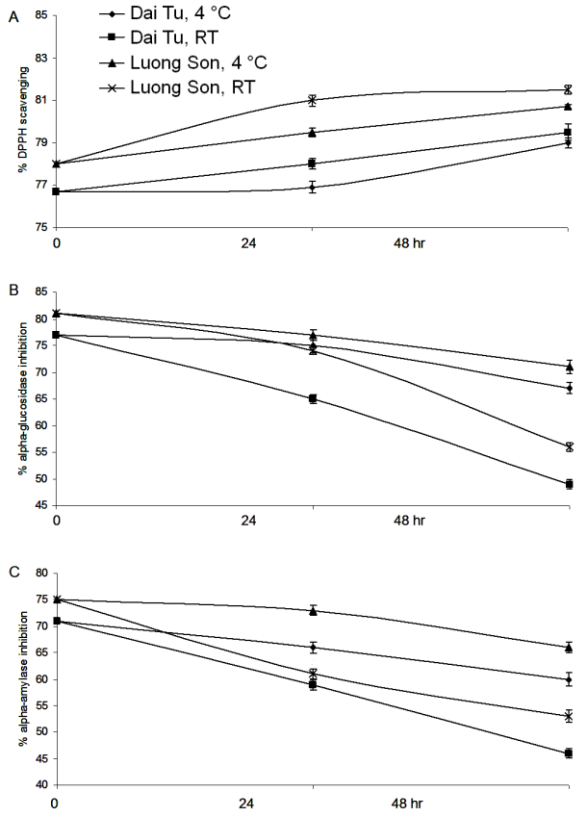

Fig. 1: Changes in DPPH scavenging (A), α-glucosidase (B) and α-amylase (C) inhibitory activities of the fresh old tea leaves during storage. Bars represent standard deviations

Old tea leaves, which are not used in the tea production, are considered as an important source of antioxidant basing on its high phenolic content, especially catechin constituents. Although this material has been used for food additive purpose in several countries, the quality has not been well studied. This paper is the first report on the evaluation of the changes in EGCG, total polyphenol contents as well as the DPPH scavenging, α-glucosidase and α-amylase inhibitory activities of the old tea leaves during storage. The results also showed the possibility of old tea leaves utilization as materials for several industrial food productions.

Acknowledgement. *This work is supported by a grant from the Vietnam National Foundation for Science and Technology Development (NAFOSTED-104.01-2011.54) to one of the authors (N.T.D.).*

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