

Variations in Antioxidant Compound Contents and Antioxidant Activity during Processing of Traditional Chinese Green Tea

SHEN Lin¹, LIU Kai-lang¹, DENG Li-li¹, SHENG Ji-ping^{1,2,*}

(1. College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China

2. School of Agricultural Economics and Rural Development, Renmin University of China, Beijing 100872, China)

Abstract: This research was conducted to investigate variations in the contents of total phenols, total flavonoids, and ascorbic acid, trolox equivalent antioxidant capacity (TEAC) and anti-auto-oxidation activity in traditional Chinese green tea at different stages of processing. The total phenol content in fresh tea leaves was 119.91 mg/g of dry weight and decreased to 100.33 mg/g in green tea. Similar trend was observed for total flavonoid content, which decreased from 2.90 mg/g to 2.04 mg/g. Moreover, both classes of compounds decreased mainly at the moisture regain stage. In addition, a gradual decrease (from 4.17 mg/g to 2.95 mg/g) in ascorbic acid content was observed. Fresh tea leaves showed a slight decrease in TEAC (from $(524.94 \pm 18.68) \mu\text{mol/g}$ to $(487.03 \pm 17.97) \mu\text{mol/g}$) after being processing into green tea. No linear relationship was observed between TEAC and total phenol or total flavonoid contents ($R^2 = 0.19$, and $R^2 = 0.01$, respectively). The anti-auto-oxidation activity of green tea was higher than that of fresh tea leaves and the inhibitory rate increased from 52.45% to 78.12%. Therefore, the contents of the main antioxidant compounds decreased and the antioxidant activity changed slightly during the processing of traditional Chinese green tea.

Key words: green tea; processing; phenolics; flavonoids; antioxidant capacity

中国绿茶传统加工过程中抗氧化物质含量及抗氧化活性的变化

申琳¹, 刘开朗¹, 邓丽莉¹, 生吉萍^{1,2,*}

(1. 中国农业大学食品科学与营养工程学院, 北京 100083;

2. 中国人民大学农业与农村发展学院, 北京 100872)

摘要: 研究中国绿茶传统加工过程不同工艺阶段茶汤总酚、总黄酮、抗坏血酸含量和抗氧化能力的变化。加工成品的总酚含量从鲜叶的 119.91mg/g(以干质量计)下降到 100.33mg/g, 总黄酮含量也有相似的变化趋势, 从 2.90mg/g 下降到 2.04mg/g, 且这两种成分的降低主要发生在“回潮”阶段。此外, 加工过程中茶汤抗坏血酸含量也逐渐下降(从 4.17mg/g 降至 2.95mg/g)。尽管经过剧烈的加工, 鲜叶总抗氧化能力(TEAC)仅出现轻微降低, TEAC 从 $(524.94 \pm 18.68) \mu\text{mol/g}$ 降至成品中的 $(487.03 \pm 17.97) \mu\text{mol/g}$, 但总酚和总黄酮与 TEAC 无线性关系($R^2=0.19$, $R^2=0.01$), 而 DPPH 自由基与总酚和总黄酮有较好的线性关系($R^2=0.77$, $R^2=0.56$)。此外, 加工过程中茶叶抗氧化能力增强(抑制率从 52.45% 增加至 78.12%)。表明中国绿茶传统加工过程导致成品茶汤主要抗氧化物质含量的减少, 但仅导致其抗氧化能力的轻微改变。

关键词: 绿茶; 加工; 总酚; 类黄酮; 抗氧化活性

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The consumption of tea has been associated with reduced risk of major diseases, including coronary heart disease, stroke and cancer^[1-3]. This function has been attributed to the polyphenols especially the flavonoids in tea, which possess a high antioxidant power which can protect cells against the adverse effects of reactive oxygen species.

Tea contains a large amount of catechins, a group of active flavonoids, and has become the focus of many studies^[4].

Traditionally, tea can be classified as green, yellow, white and black, etc. Green teas are produced by inactivating the enzymatic oxidation of phenolics found in the tea leaf^[5]. Catechins constitute about 20% — 30% of the weight of the

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作者简介: 申琳(1964—), 男, 副教授, 博士, 主要从事食品生物工程研究。E-mail: shen5000@cau.edu.cn

* 通信作者: 生吉萍(1967—), 女, 教授, 博士, 主要从事果蔬采后生理与生物技术研究。E-mail: pingshen@cau.edu.cn

tea leaf^[6]. Almost all of the characteristics of manufactured tea, including its taste, color, aroma, and antioxidant capacity are associated directly or indirectly with modifications to the catechins. Tea catechins undergo many chemical changes such as oxidation and epimerisation during the course of the processing processes. Studies have been undertaken on the oxidative conversion of catechins to theaflavins and thearubigins during black tea manufacture^[7]. In green tea processing, it is believed that non-enzymatic oxidation of polyphenols could be important during the drying process, as found in cocoa beans^[8]. Considering green tea catechins are not equally active^[9], it is reasonable to speculate that processing play an very important role on the chemical composition and subsequent antioxidant capacity of the final tea product.

The manufacture of green tea is a complex biological process, where the final quality depends on many factors, including the chemical composition of the green leaf, the extent to which the tea leaf are withered, curled and dehydrated during processing. However, little is known about the changes of antioxidant components and subsequent antioxidant capacity of tea infusions during green tea processing. The present study has been designed to investigate the changes of major antioxidants compounds, namely as ascorbic acid, phenolics and flavonoids and total antioxidant capacity of the green tea infusions during Chinese traditional processing.

1 Materials and Methods

1.1 Materials, reagents and instruments

The tea (*Camellia sinensis*) leaf used in the study was hand-plucked from the Guzhang Tea Farm in Guangxi province.

The solvents used for the extraction of tea infusion samples were analytical grade. 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonate)(ABTS), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), linoleic acid, β -carotene and Folin-Ciocalteu reagent were obtained from Sigma Chemical Co., (St Louis, MO, USA). Other chemicals were all analytical grade and were purchased from Beijing Chemical Co..

All spectrophotometric data were acquired using a UVikon spectrophotometer (Secman, France).

1.2 Samples and tea processing

The tea (*Camellia sinensis*) leaf used in the study was

hand-plucked from the Guzhang Tea Farm in Guangxi province on Oct 17, 2004. Plucking standards conformed to the normal recommended commercial practice of mostly two leaves and a bud, plus minor amounts of three leaves and a bud. 9 kg green leaf was plucked per replicate. After collection, the samples were wrapped in washed calico and packed in dry ice in polystyrene foam boxes. All samples were then delivered with dry ice by overnight flight from Guangxi to Beijing. After arrival, all the samples were sorted, and samples in good condition were subjected to Chinese traditional green tea processing.

There are six steps for Chinese green tea processing: first, "withering (WT)" that belted tea for up to 2–3 h in order to reduce the initial moisture content to approximately 70%. Second, "Shaqing (SQ)", which mean deactivation of enzymes, that the withered leaves were heated in an iron pot, 80–90 cm in diameter, and stir-fried continuously with a shovel. Third, "curling (CL)" for 30 min in which the leaves were macerated to form the tightly rolling shape. Fourth, "first firing (FF)" which would remove the water adhered to the surface of the leaves and reduced the moisture to about 40%, then leaves were laid open at room temperature for 1–2 h to make the inner moisture redistribute equally, which was called "Huichao (HC)". Last, leaves were fired for the second time at approximately 80 °C to reduce the moisture to 5%–6% ("second firing (SF)"). During each step, samples were taken. Plus the fresh tea (FT) leaves, there are seven samples in the study which named with abbreviation as mentioned above in the order of processing procedure.

1.3 Preparation of tea infusions and methanolic extract

Tea infusions were obtained by boiling 10 g samples in 300 mL of distilled water for 20 min, cooled at room temperature for 30 min and then filtered through Whatman No.1 filter paper. Supernatant were pooled for the assay of total antioxidant capacity, the DPPH radical-scavenging activity and the anti-oxidation assay.

Extraction of phenolic compounds using methanol was carried out according to our previous study^[10]. Green leaf was extracted with methanol at 25 °C for 15 h. The extracts were filtered through Whatman No.1 filter paper and the supernatants were pooled for the determination of total phenolic and flavonoid contents

1.4 Determination of ascorbic acid, total phenolic and flavonoid contents

Total L-ascorbic acid content was determined using a 2,6-dichlorophenol (DIP) titrimetric method^[11].

Total phenolics was determined by Folin-Ciocalteu reagent in alkaline medium and was expressed as gallic acid equivalents^[12]. Total flavonoids was determined by using a colorimetric method described previously^[11]. Briefly, 0.5 mL of the extract or (+)-catechin standard solution was mixed with 1.25 mL of distilled water in a test tube, followed by addition of 75 μ L of a 5% sodium nitrite solution. After 6 min, 150 mL of a 10% aluminum chloride solution was added and the mixture was allowed to stand for a further 5 min before 0.5 mL of 1 mol/L sodium hydroxide was added. The mixture was brought to 3.0 mL with distilled water and mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. The results were expressed as ($\bar{x} \pm s$) mg of (+)-catechin equivalents per gram on dry weight (DW).

1.5 Assay of total antioxidant capacity using TEAC assay

Extracts were tested by using the ABTS test system, with trolox as standard substance, and trolox equivalent antioxidant capacity (TEAC) as evaluating indicator. The TEAC value is based on the ability of the antioxidant to scavenge the radical cation ABTS⁺ by spectrophotometric analysis^[13]. The ABTS cation radical was produced by the reaction between 7 mmol/L ABTS in distilled H₂O and 2.45 mmol/L potassium persulfate, stored in the dark at room temperature for 12 h. The ABTS⁺ solution was then diluted with PBS(pH 7.4) to an absorbance of 0.70 ± 0.01 at 734 nm and equilibrated at 30 °C. The reaction was initiated by the addition of 3 mL of diluted ABTS to 20 μ L of each extract, and the decrease in the absorbance at 517 nm was monitored using a Secman UvikonXL spectrophotometer in time-drive model until the reaction reached the steady state. Determinations were repeated three times for each extract. The percentage inhibition of absorbance at 734 nm was calculated for each extract relative to a blank absorbance and was plotted as a function of standard Trolox.

1.6 DPPH radical-scavenging activity

The free radical scavenging activity of extracts and their solvents were measured by the DPPH method proposed by Brand-Williams, et al^[14]. Briefly, DPPH solution (1 mmol/L in methanol) was then diluted with ethanol to an absorbance of 0.70 ± 0.01 at 517 nm. A solution (20 μ L) of the extract was added to 3 mL of diluted DPPH solution, and the decrease in the absorbance at 517 nm was monitored using a Secman UvikonXL spectrophotometer in time-drive model until the reaction reached the steady state.

$$\text{Inhibition rate } \% = (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100$$

1.7 Anti-antioxidation assay

The anti-antioxidation capacity was assayed by the method described by Pratt^[15]. Quantities of linoleic acid (20 mg) and Tween-20 (200 mg) were mixed and placed in a flask, before the addition and solution of 2 mg β -carotene in 20 mL chloroform. After removal of chloroform using with a rotary evaporator at 50 °C, 50 mL of distilled water saturated with oxygen for 30 min was added. Aliquots (0.8 mL) of the β -carotene emulsion and 0.1 mL of extracts were placed in capped culture tubes and mixed well. The tubes were immediately placed in a water-bath and incubated at 50 °C. The absorbance at 470 nm was recorded at an intervals of 20 min for 6 times (up to 100 min). Distilled water was used as blanks. The same of the formula of the inhibition rate as Title 1.6.

1.8 Statistical analysis

All analyses were performed in triplicate. The data were expressed as $\bar{x} \pm s$ deviations and analyzed by SPSS (Version 10.1). Differences between means at the 5% level were considered significant.

2 Results and Analysis

2.1 Ascorbic acid, total phenolics and flavonoids contents of green tea infusions during processing

Special attention has been paid to antioxidants, especially phenolics, in tea which possess high antioxidant potential. The changes in total phenolics, total flavonoids and ascorbic acid concentrations in green tea infusions during Chinese traditional processing were shown in Table 1.

Table 1 Total phenolic, total flavonoid and ascorbic acid contents in different green tea infusions ($\bar{x} \pm s$, $n=3$)

Samples	Total phenolics/(mg/g)	Total flavonoids/(mg/g)	Ascorbic acid/(mg/g)
FT	119.91 ± 5.14^b	2.90 ± 0.17^a	4.17 ± 0.06^a
WT	108.95 ± 6.89^c	2.82 ± 0.16^a	2.95 ± 0.10^b
SQ	84.44 ± 1.38^c	1.97 ± 0.08^c	1.86 ± 0.05^c
CL	94.66 ± 4.37^d	1.73 ± 0.11^d	1.41 ± 0.08^d
FF	145.59 ± 4.30^a	2.58 ± 0.09^b	1.17 ± 0.06^c
HC	48.48 ± 1.08^f	0.90 ± 0.07^e	1.14 ± 0.05^c
SF	100.33 ± 2.06^{cd}	2.04 ± 0.05^c	1.12 ± 0.06^c

Note: Values with different letters within a column are significantly different ($P < 0.05$).

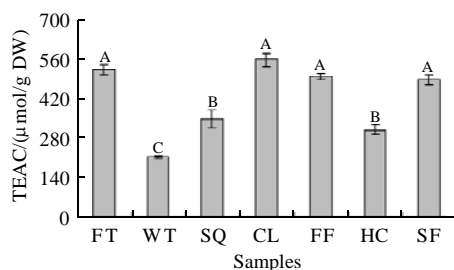
Total phenolics in green tea infusions showed significant variation as affected by processing. Compared with fresh green leaf, total phenolic content decreased from 119.91 mg/g in fresh leaf to 100.33 mg/g, of final product, while much of decrease occurred during two steps, “Shaqing” and “Huichao”.

Total flavonoid content decreased from 2.90 mg/g, in fresh leaf to 2.04 mg/g, of final product, which showed a similar pattern with that of total phenolic content. In tea during processing, partly because they both come from phenylpropanoid metabolic pathway.

In this study, ascorbic acid concentrations were found to decrease through the whole processing. Ascorbic acid concentration showed a decrease from 4.17 mg/g in fresh leaves to 2.95 mg/g in withered leaves. This behavior can be attributed to the ascorbic acid to reduce the radicals even in the early stages of the oxidation^[16]. During the following thermal treatments, ascorbic acid concentration decreased to 1.12 mg/g in final product, which might be explained by the fact that ascorbic acid is a heat-unstable vitamin.

2.2 ABTS test system

To access the effect of processing on antioxidant capacity of green tea, samples were subjected to the ABTS test system, with TEAC as evaluating indicator. Fig.1 was shown that tea had very high antioxidant capacity, and sample CL has the highest TEAC (560.10 ± 22.69 $\mu\text{mol/g}$). Despite the intense operations, only a slight decrease in total antioxidant capacity was seen from fresh leaf (TEAC (524.94 ± 18.68) $\mu\text{mol/g}$) to final product (TEAC (487.03 ± 17.97) $\mu\text{mol/g}$).



Values with different letters within a column are significantly different ($P < 0.01$); Same as the follow.

Fig.1 Antioxidant capacities of different green tea infusions, assessed by TEAC assay ($\bar{x} \pm s$, $n = 3$)

It is noticeable that nonlinear correlations between phenolics and flavonoids concentrations and antioxidant capacities were observed ($R^2=0.19$, $R^2=0.01$, respectively). Combined with previous research, this behavior can be explained by two facts, on one hand, this can be attributed to the changes in the oxidation state, instead of concentration of phenolics. During green tea processing, green tea leaves were subjected to intensely operations, such as repeated curling and firing at about 80 °C, which were believed to cause enzymatic and non-enzymatic oxidation to phenolic

compounds. Although oxidations have been widely proved to cause a progressive decrease in poly-phenol antioxidant properties, polyphenols with an intermediate oxidation state can exhibit higher radical scavenging activity than the non-oxidized ones^[17]. It has been observed that catechin that had undergone chemical oxidation, proceeding with a lower rate, shows a remarkable increase in its chain breaking activity^[18].

On the other hand, it should be taken into consideration that food compounds can also react with each other. During thermal treatments, food can be subjected to other chemical changes such as resulting from the Maillard reaction^[19]. During the processing, leaves were subjected to repeated curling, which caused the breakdown of some cell, and then to firing at 80 °C for about 60 min. Those operations can certainly induce the Maillard reaction in green tea leaf, which is supported by the changes in the leaf color from brownish green before the fire and brown after. The development of the Maillard reaction causes the formation of compounds with antioxidant properties, which minimized the loss of natural antioxidants, even enhancing the overall antioxidant properties of the product^[20].

2.3 DPPH radical-scavenging capacity

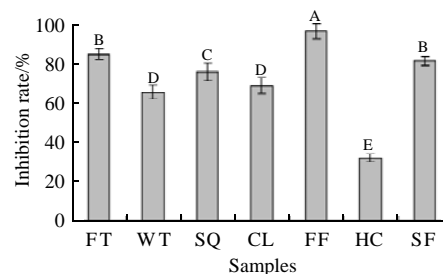


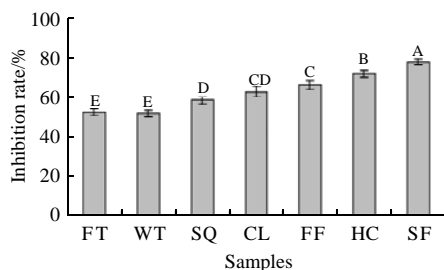
Fig.2 DPPH radical-scavenging capacities of different green tea infusions ($\bar{x} \pm s$, $n = 3$)

The DPPH free radical is a stable free radical, which has been widely used as tool to estimate antioxidant capacity^[21]. Fig.2 illustrated the inhibition rates of the DPPH radical due to the scavenging capacity of different green tea infusions. There was significant difference ($P < 0.01$) between sample HC and others in DPPH radical- scavenging capacity. In contrast with results obtained in TEAC test, this pattern is better correlated with that of total phenolic and flavonoid contents ($R^2=0.77$, $R^2=0.56$, respectively).

In this study, both ABTS and DPPH test system were used to determine the total antioxidant capacity of green tea infusions. Recent investigations showed there were differences between the test systems for the determination of antioxidant activity^[22-23]. So it is clear that there is not a single

method that can give a comprehensive prediction of antioxidant efficacy, thus the use of more than one method is recommended.

2.4 Results of anti-antioxidation assay



Distilled water was used as control.

Fig.3 Antioxidant capacities of different green tea infusions, measured by bleaching of linoleic acid-carotene emulsion ($\bar{x} \pm s$, $n = 3$)

Fig.3 indicated the decrease of the absorbance of β -carotene when the presence of different green tea infusions. All of the green tea infusions showed a strong inhibition on bleaching of β -carotene. Anti-antioxidation capacities of samples increased though the whole processing, with the inhibition increasing from 52.45% to 78.12%. It showed Chinese traditional tea processing increased anti-antioxidation capacity of green tea.

3 Conclusions

This study suggests that Chinese traditional tea processing causes sharply decrease in total phenolic and flavonoid contents ($P < 0.05$), besides, a significant decrease of the concentrations of ascorbic acid were found through the whole processing ($P < 0.01$). However, despite the intense operations, only a slight decrease in total antioxidant capacity, assayed by ABTS and DPPH test systems, was seen from fresh leaf to final product. In addition, the processing was found to increase anti-antioxidation capacity of green tea.

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