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Original Article

Relative content of gallic acid over 5-galloylquinic acid as an index for the baking intensity of oolong teas

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ABSTRACT

Phenolic compounds in a series of old oolong teas prepared by baking annually were monitored and compared. The results showed that the relative content of gallic acid over 5-galloylquinic acid was subsequently elevated during this preparatory process. To reveal the effect was mainly resulted from baking or aging, two sets of oolong teas were collected and examined; one set was generated from fresh oolong tea via continually daily baking and the other set was composed of aged oolong teas with no or light baking in the storage period. The relative content of gallic acid over 5-galloylquinic acid was observed to be subsequently elevated when oolong tea was continually baked at 90, 100, 110, and 120 °C for 8 h day after day. In contrast, the relative contents of gallic acid over 5-galloylquinic acid in aged oolong teas with no or light baking were found to be similar to or slightly higher than that in fresh oolong tea. The results suggest that the relative content of gallic acid over 5-galloylquinic acid seems to be a suitable index for the baking intensity of oolong tea in different preparations.

1. Introduction

Tea is regarded as one of the most consumed beverage in the world [1–3]. In the past few decades, several phenolic compounds, such as epigallocatechin-3-gallate (EGCG), gallic acid, strictinin, and teaghrelin, in tea infusion have been demonstrated to provide a variety of beneficial functions to human health [4–11]. According to the different ways of processing, tea is mainly classified into three basic types, green tea (unfermented), oolong tea (partially fermented), and black tea (fully fermented), where "fermentation" refer to natural browning reactions caused by oxidative enzymes in the cells of tea leaves [12,13]. Oolong tea is produced predominantly in Fujian and Guangdong of China as well as in Taiwan. In the past century, different types of oolong teas have been innovatively produced via versatile processes in Taiwan, and are highly appreciated by Taiwanese due to their special taste and flavor [14].

Among different types of oolong teas, old oolong tea has become more and more popular in Taiwan as well as in China recently [15]. Old oolong tea is commonly referred to those

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oolong teas stored for a certain period of time, e.g., more than five years, and may be occasionally refined by baking during the storage [14]. Empirically, the longer oolong teas are stored and further oxidized gradually, the better they are in terms of taste. Furthermore, oolong teas with relatively high degrees of fermentation are thought to have a better chance to be converted into superior old oolong teas. A major type of old oolong teas commonly practiced in Taiwan utilized periodical baking refinement at least once a year during the aging process. Besides the fermentation process, long-term storage (aging) and professional baking are typically regarded as two major factors for the quality control in the preparation of old oolong teas [16]. As expected, different combinations of aging and baking processes have led to the commercial production of diverse old oolong teas with distinct tastes empirically [14]. According to liquid chromatography tandem mass spectrometry (LC/MS/MS) and gas chromatography/mass spectrometry (GC/MS) analyses, the phenolic and volatile compounds of oolong teas were evidently altered under the baking and aging processes [17,18].

As significant difference in chemical constituents was observed between fresh oolong teas and old oolong teas, we wondered if any alteration of chemical constituents in oolong teas could be assigned as a characteristic index for the effects of the baking and aging processes in the tea conversion. In this study, a series of old oolong teas prepared by baking annually were obtained from a local tea manufacturer in Taiwan, and their phenolic compounds were analyzed and compared. Strikingly, the relative content of gallic acid over 5-galloylquinic acid was found to be subsequently elevated during the conversion process of old oolong tea. To reveal the elevation of gallic acid over 5-galloylquinic acid was mainly resulted from baking or aging, two sets of oolong teas were collected and examined; one was prepared from fresh oolong tea by daily baking and the other was composed of aged oolong teas with no or light baking in the storage period. The results indicated that baking intensity seemed to be responsible for the elevation of gallic acid over 5-galloylquinic acid in old oolong teas.

2. Methods

2.1. Chemicals and materials

All chemicals were purchased from E. Merck Co. (Merck KGaA, Darmstadt, Germany) unless stated otherwise. High performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Acetic acid (99.7%) was obtained from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA). Purified water was afforded by a Millipore clear water purification system (Direct-Q, Millipore, Billerica, MA, USA).

2.2. Tea samples

Fresh Chin-shin oolong tea was prepared from tea plants (*Camellia sinensis* L.) grown in Mountain Ali, Chayi County, Taiwan in 2015. A series of old oolong teas prepared by baking annually and old oolong teas with no or light baking (a few times at temperature lower than 100 °C) in the storage period were gifts from local tea manufacturers. To obtain a set of baked oolong teas, fresh oolong tea was subjected to daily baking for 8 h at 90, 100, 110, and 120 °C subsequently by a drier composed of two parts, an electronic heater made of stainless steel (60 cm in diameter) and a tea container with a steel net at the bottom (58 cm in diameter) encircled with dry bamboo sheet (40 cm in height).

2.3. Preparation and HPLC analysis of tea infusions

Tea infusions were prepared by adding 20 mL of boiling water to 1 g of fresh, old or baked oolong teas for 15 min. After cooling to room temperature, the brew was filtered through a 0.22 μm polyvinylidene difluoride (PVDF) membrane filter (Pall Corporation, Glen Cove, NY) for the following analysis. Chemical constituents in oolong tea infusions were analyzed by HPLC system coupled to a 600E photodiode array detector (Waters Corporation, Milford, MA), and separation was performed on the Syncronis C18 column (4.6 × 250 mm inner diameter, 5 μm, Thermo Scientific, Waltham, MA, USA). The separated condition of HPLC analysis was modified according to Shih et al. [19]. The mobile phase consisted of (A) water containing 0.5% acetic acid and (B) acetonitrile. The program for gradient elution started at 95% solvent A and 5% solvent B, increased linearly to 77% solvent A and 23% solvent B in 70 min. The column was maintained at room temperature and the injection volume was 5 μL at a flow rate of 1 mL/min. The ultraviolet (UV) absorbance detection wavelength was set at 280 nm. Epigallocatechin-3-gallate (EGCG), caffeine, gallic acid (GA), and 5-galloylquinic acid (5GA) shown in the HPLC profiles of this study were identified according to the same procedure as described previously [10].

2.4. Detection of UV-Vis spectra of tea infusions

To detect UV–Vis spectra, tea infusions prepared as described above were cooled down to room temperature for 30 min and filtrated through the PVDF membrane filter. The filtrates of 200 μL were loaded onto a 96-well UV transparent plate (Corning, Corning, USA), and UV-vis spectra were scanned from 250 to 600 nm by the Infinite® M200 pro microplate reader (TECAN, Austria, Switzerland).

3. Results

3.1. Tea leaves and infusions of a series of old oolong teas baked annually

To examine the effects of preparatory processes on the outward appearance of old oolong teas baked annually, tea leaves and infusions of fresh, 10-year, 15-year, and 20-year old oolong teas were compared (Fig. 1). In comparison of the fresh oolong tea and old oolong teas, the dried tea leaves seemed to transform from dark yellow green to dark brown or light black after the preparatory processes. Tea infusion color of fresh oolong tea was yellow green while that of old oolong teas was brown red. Tea leaves of old oolong teas as well as those of fresh oolong tea could fully expand to their original sizes when they

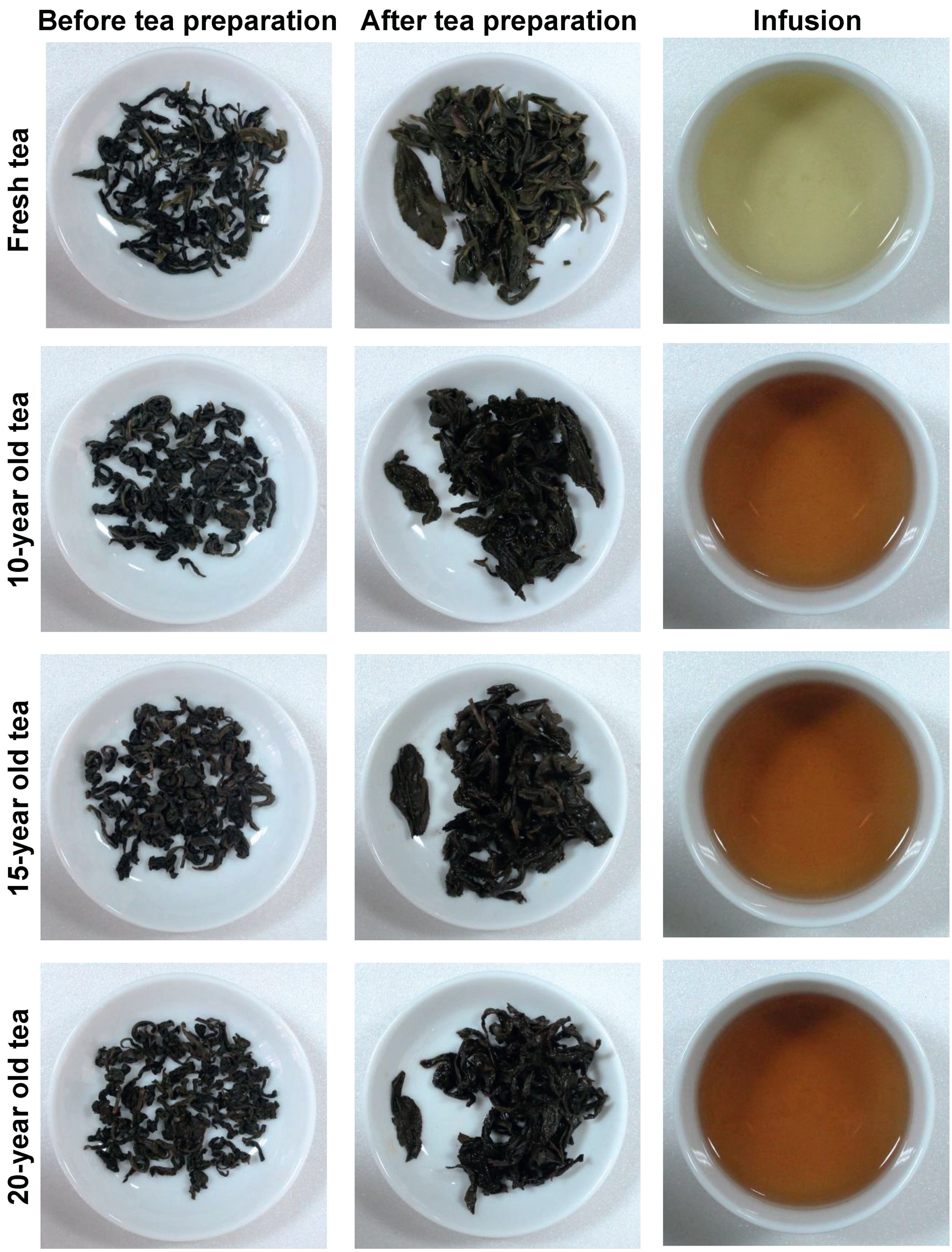

Fig. 1 – Tea leaves and infusions of fresh, 10-year, 15-year, and 20-year old oolong teas. Tea leaves were shown before and after tea preparation.

absorbed hot water in a regular tea preparation. However, the tea leaves of old oolong teas looked darker in color, slightly shrank and became less flexible in contrast with the soft tea leaves of fresh oolong tea after tea preparation. Not much difference was observed among the three old oolong teas, in terms of color and shape of dried tea leaves, appearance of leaves after tea preparation, and color of tea infusion.

3.2. *Elevation of the relative content of gallic acid over 5-galloylquinic acid in the preparation of old oolong teas baked annually*

To detect the changes of phenolic compounds in the preparation of old oolong teas, tea infusions of the fresh oolong tea and the three old teas were subjected to HPLC analysis (Fig. 2).

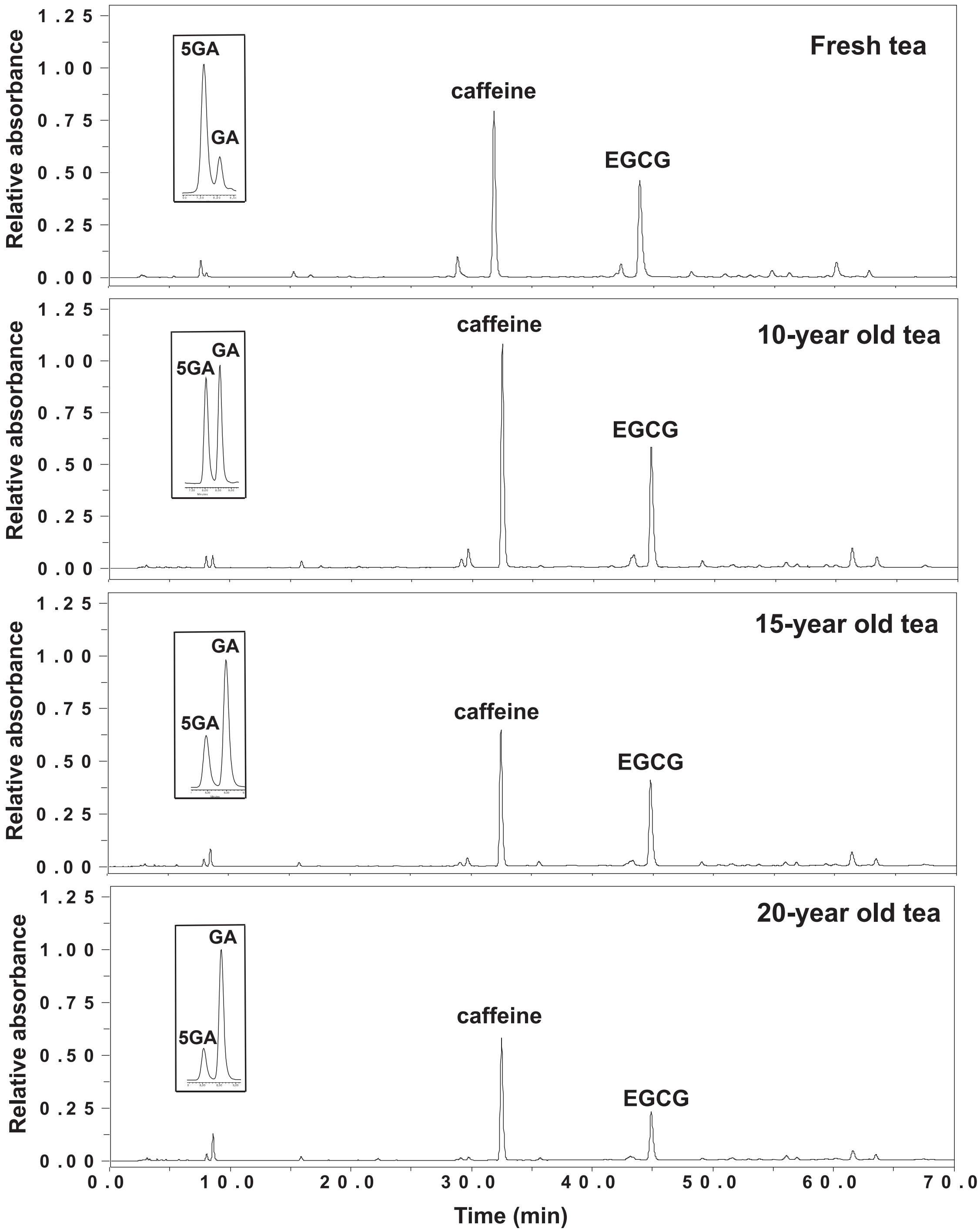

Fig. 2 – HPLC profiles of fresh, 10-year, 15-year, and 20-year old oolong teas at 280 nm. Chemical constituents in the infusions of oolong teas were separated by HPLC (0–70 min). The peaks of 5-galloylquinic acid (5GA), gallic acid (GA), caffeine, and epigallocatechin-3-gallate (EGCG) were indicated. The retention times of 5-GA, GA, caffeine, and EGCG are approximately 7.5, 8.0, 33, and 45 min, respectively.

Among the three old oolong teas, the contents of caffeine and the major polyphenolic compounds, such as EGCG, were found to be subsequently reduced in these tea samples along with the preparatory processes of old oolong teas. Moreover, the content of 5-galloylquinic acid (5-GA) was drastically decreased while that of gallic acid (GA) was substantially increased in these samples in response to the tea conversion. Taken together, the relative content of gallic acid over 5-galloylquinic acid was significantly elevated in the preparatory processes of old oolong teas.

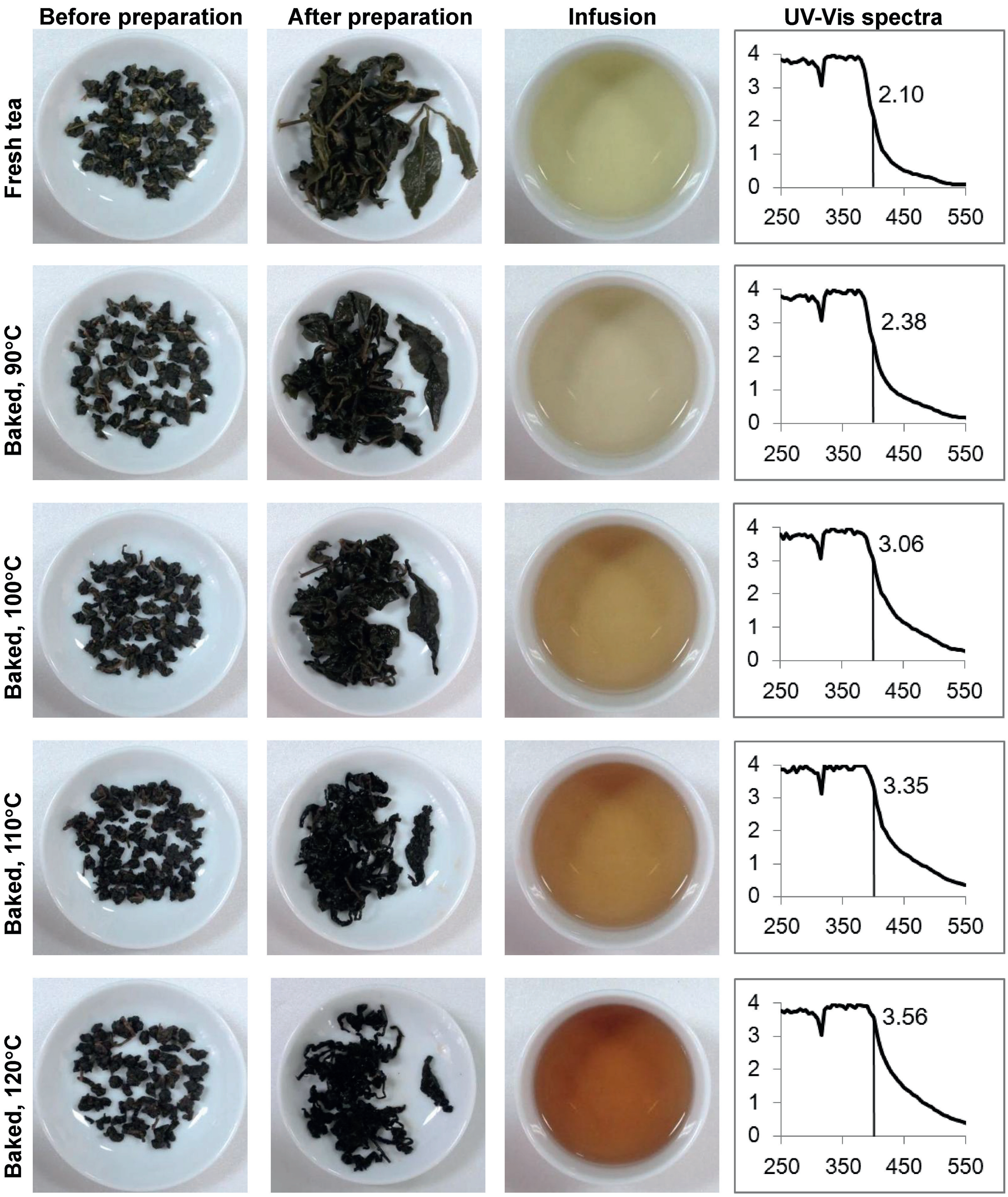

Fig. 3 – Tea leaves and infusions of fresh oolong tea and oolong teas baked daily for 8 h at 90, 100, 110, and 120 °C. Tea leaves were shown before and after tea preparation. UV-Vis spectra of tea infusions were shown on the right panels, and the absorption values at 400 nm were indicated in the diagrams.

3.3. *Preparation and examination of fresh oolong tea baked daily*

To monitor if the significant elevation in the relative content of gallic acid over 5-galloylquinic acid was resulted from baking, a set of baked oolong teas were generated from fresh oolong tea via continually daily baking for 8 h at 90, 100, 110, and 120 °C subsequently (Fig. 3). During this baking process, the color of tea granules gradually converted from dark yellow green to light black; the tea infusion color progressively changed from yellow green to brown red; the leaves after tea preparation apparently

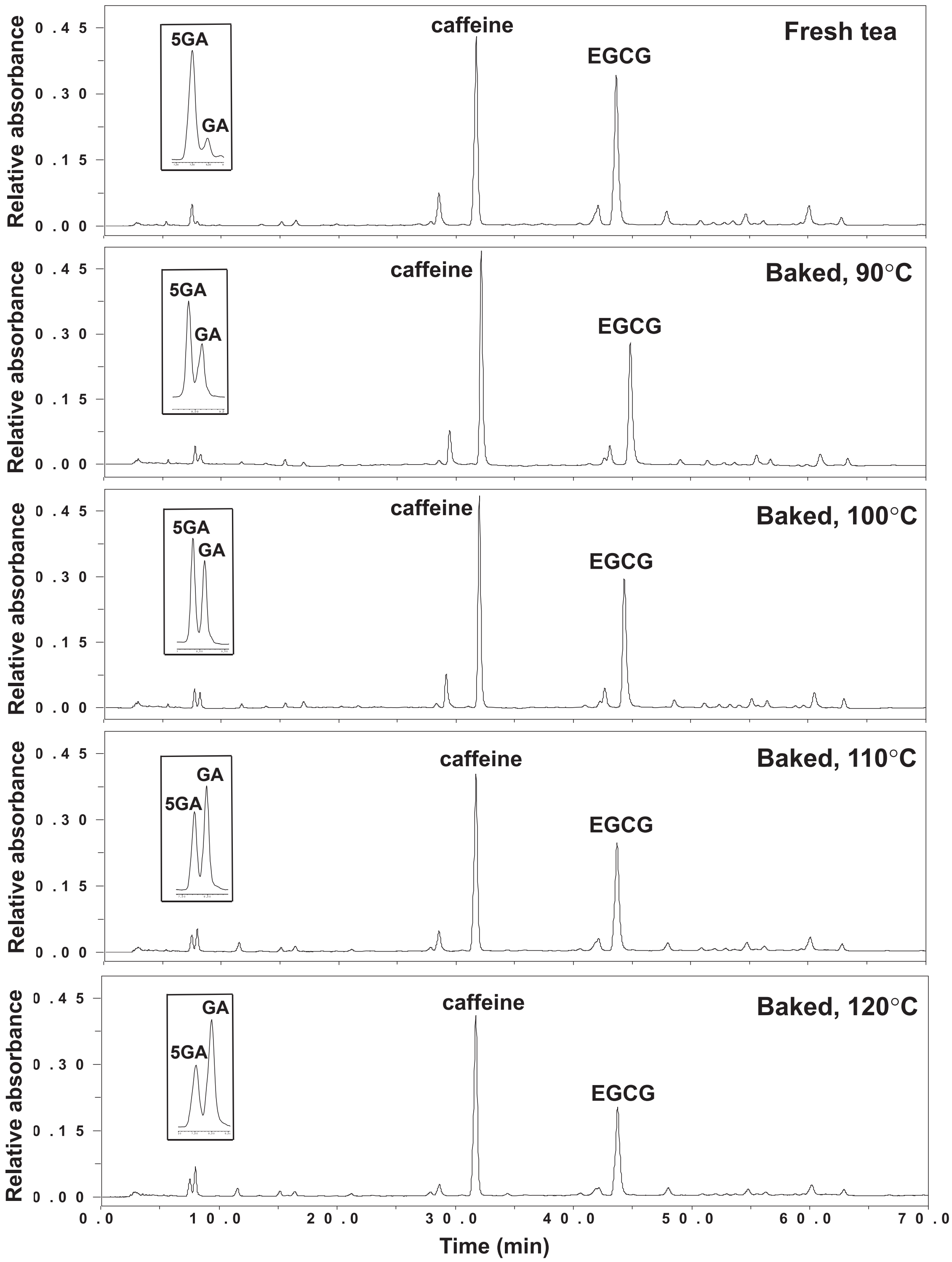

Fig. 4 – HPLC profiles of fresh oolong tea and oolong teas baked daily for 8 h at 90, 100, 110, and 120 °C. Chemical constituents in the infusions of oolong teas were separated by HPLC (0–70 min). The peaks of 5-galloylquinic acid (5GA), gallic acid (GA), caffeine, and epigallocatechin-3-gallate (EGCG) were indicated.

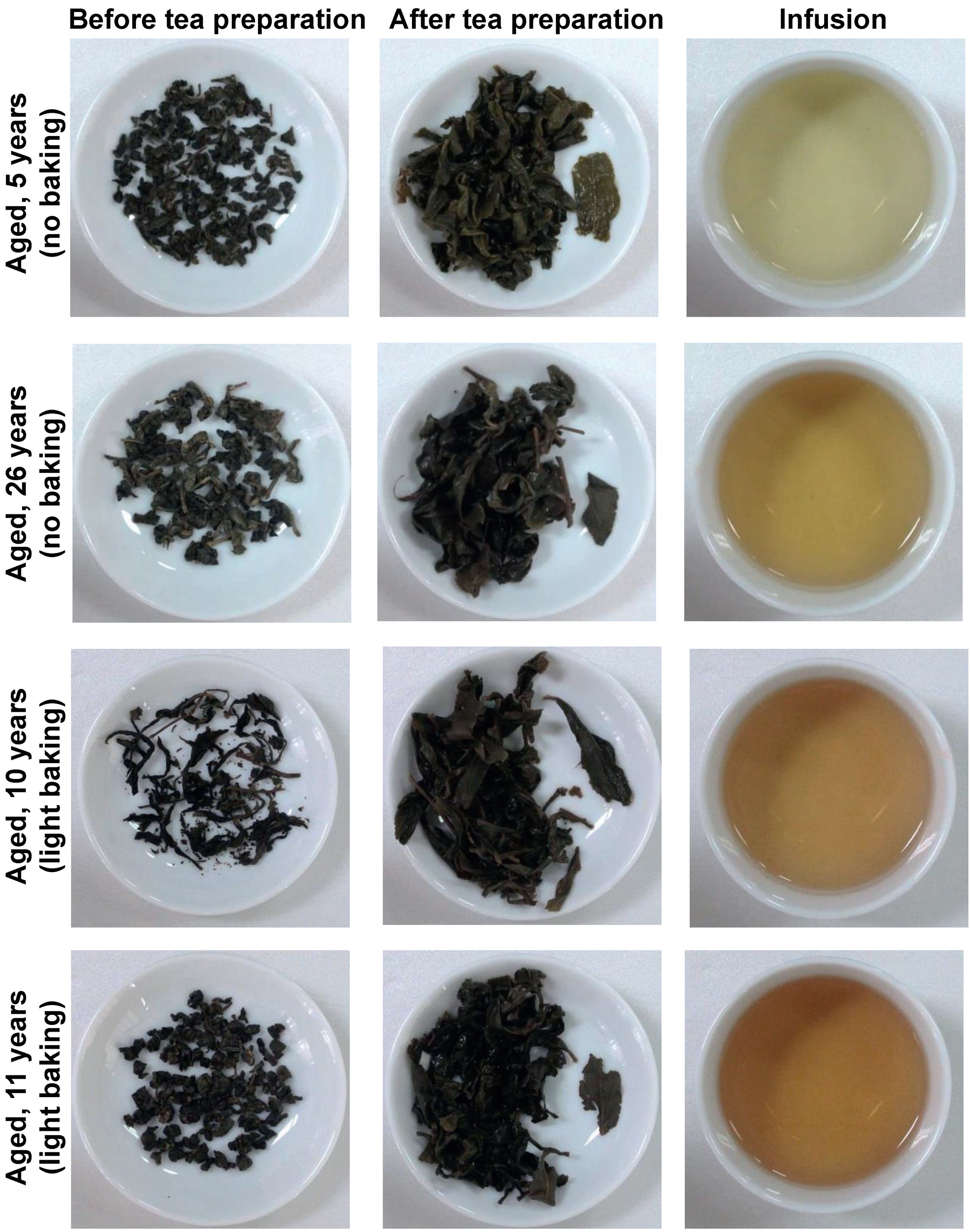

Fig. 5 – Tea leaves and infusions of two aged oolong teas without baking and two aged oolong teas with light baking. Tea leaves were shown before and after tea preparation.

shrank and could no longer fully expand to their original sizes and their color transformed from yellow green to black. The color change in tea infusion was concurrently observed in the UV–Vis spectra, particularly at 400 nm. No apparent difference was observed in the content of caffeine while that of EGCG was subsequently reduced in the baking process with elevated temperature (Fig. 4). Furthermore, the content of 5-galloylquinic acid was nearly unaltered or subtly lessened when the baking temperature was lower than 100 °C, but drastically declined at baking temperature higher than 110 °C. In contrast, the content of gallic acid was subsequently increased in the whole process of baking conversion. The results indicated that baking was able to elevate the relative content of gallic acid over 5-galloylquinic acid in oolong tea. A simple quantitative analysis showed that the relative content (area under the peak) of gallic acid over 5-galloylquinic acid subsequently changed after daily baking: 0.19 for fresh tea, 0.69 for baked at 90 °C, 0.84 for baked at 100 °C, 1.38 for baked at 110 °C, and 1.73 for baked at 120 °C.

3.4. *Examination of aged oolong teas with no or light baking during the storage period*

To evaluate the effects of aging on the relative content of gallic acid over 5-galloylquinic acid, two aged oolong teas without baking and two aged oolong teas with light baking in the storage period were examined (Fig. 5). For the aged oolong teas

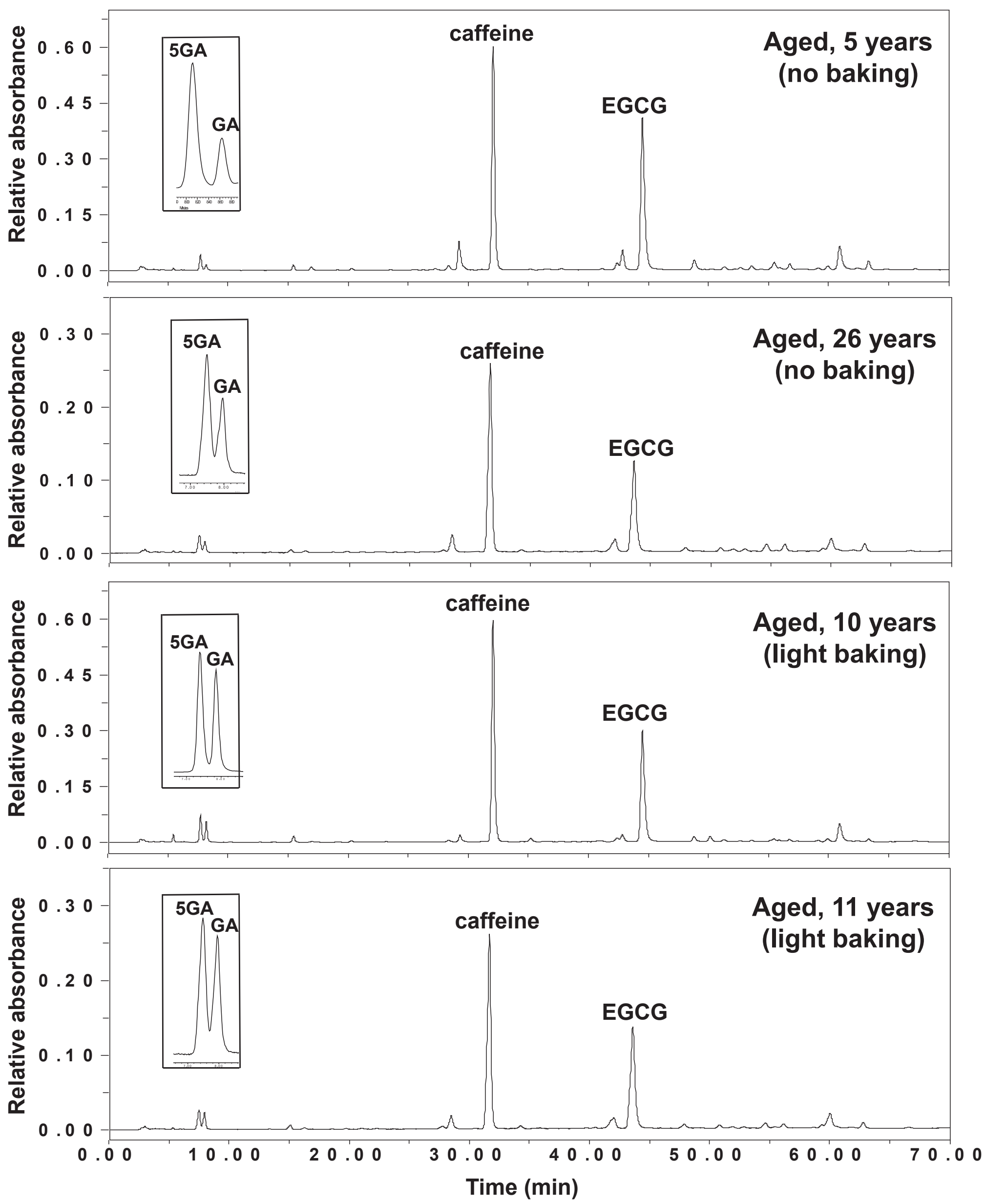

Fig. 6 – HPLC profiles of two aged oolong teas without baking and two aged oolong teas with light baking. Chemical constituents in the infusions of oolong teas were separated by HPLC (0–70 min). The peaks of 5-galloylquinic acid (5GA), gallic acid (GA), caffeine, and epigallocatechin-3-gallate (EGCG) were indicated.

without baking, the color of dried tea leaves was dark yellow green, the tea infusion color was golden yellow, and the leaves after tea preparation could fully expand to their original sizes with yellow green color; all examined properties were similar to those of fresh oolong tea. For the aged oolong teas with light baking, the color of dried tea leaves was dark brown, the tea infusion color was yellow brown, and the leaves after tea preparation could also fully expand to their original sizes with light yellow brown color. The relative contents of gallic acid over 5-galloylquinic acid in aged oolong teas with no or light baking were found to be similar to or slightly higher than that in fresh oolong tea (Fig. 6). The slight elevation of gallic acid over 5-galloylquinic acid in aged oolong teas with light baking was comparable to that in baked oolong tea at 100 °C (Fig. 4). The results indicated that long-term storage (aging) was not able to significantly elevate the relative content of gallic acid over 5-galloylquinic acid in oolong tea.

3.5. *Examination of an oolong tea with high degree of fermentation*

To evaluate the effects of fermentation on the relative content of gallic acid over 5-galloylquinic acid, an oolong tea with high degree of fermentation (close to the fully fermented black tea) was examined (Fig. 7). As expected, the tea infusion color was orange red and the major polyphenolic compounds, such as EGCG, were significantly reduced in this highly fermented tea. While the content of EGCG was significantly reduced, the content of gallic acid was not found to be significantly increased as observed in the old oolong teas. Moreover, the content of 5-galloylquinic acid was not reduced in this tea under the process of high degree of fermentation. Taken together, the relative content of gallic acid over 5-galloylquinic acid in this highly fermented tea was similar to that in fresh oolong tea or aged oolong teas without baking.

4. Discussion

In this study, the analysis of phenolic compounds in a series of old oolong teas prepared by baking annually revealed that the relative contents of gallic acid over 5-galloylquinic acid in these tea samples were subsequently elevated during the conversion process of old oolong tea. Further analysis of two sets of oolong teas, one prepared from fresh oolong tea by daily baking and the other composed of aged oolong teas with no or light baking in the storage period showed that baking intensity instead of long-term storage was responsible for the elevation of gallic acid over 5-galloylquinic acid in the conversion of old oolong teas. Therefore, it is suggested that the

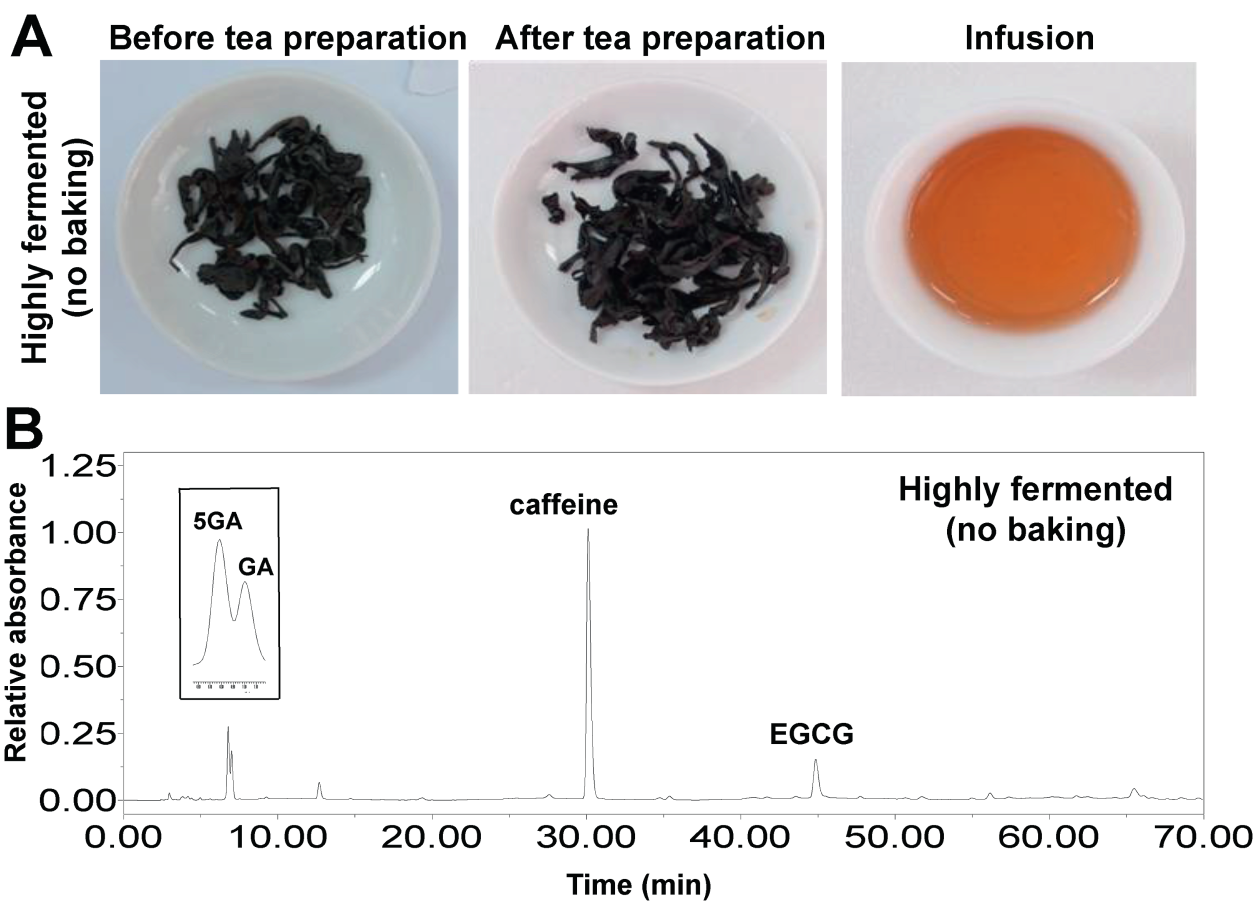

Fig. 7 – (A) Tea leaves and infusion of a highly fermented oolong tea. Tea leaves were shown before and after tea preparation. (B) HPLC profile of a highly fermented oolong tea. Chemical constituents in the tea infusion were separated by HPLC (0–70 min). The peaks of 5-galloylquinic acid (5GA), gallic acid (GA), caffeine, and epigallocatechin-3-gallate (EGCG) were indicated.

relative content of gallic acid over 5-galloylquinic acid may be used to serve as an index for the baking intensity of oolong tea in different preparations. In a more detailed examination, some other minor compounds, such as peaks at 22, 36 and 43 min might also be potential candidates as indices for baking and aging of oolong tea; of course, further investigation is indispensable for the verification on the usefulness of these peaks.

It has been proposed that 5-galloylquinic acid is an umami-enhancing constituent in oolong tea as it is able to elevate the umami intensity of glutamate proportionally [16]. In this aspect, maintenance of 5-galloylquinic acid as well as free amino acids in aged teas without baking seems to provide a higher umami taste. The contents of phenolic compounds including 5-galloylquinic acid in oolong teas remained relatively stable during the aging process, but were significantly altered by fierce baking at 120–140 °C for three days [15]. In comparison with the polyphenolic contents in fresh oolong teas, the contents of major polyphenolic compounds, catechins, such as EGCG were significantly reduced while the content of gallic acid was substantially increased in the old oolong teas prepared by periodical baking refinement [14,15]. Similar chemical alteration was observed in green teas under heat treatments [20,21]. Gallic acid possesses anti-bacterial, anti-viral, anti-diabetic, and anti-free radical, activities as well as cardiovascular protective effects and repression of tumor proliferation, angiogenesis and invasion [22–25]. Moreover, gallic acid was identified as an umami-enhancing compound in the Japanese powdered green tea [26]. Therefore, the increase of gallic acid in oolong teas may improve their taste as well as enhance the biological activities beneficial for human health.

The accumulation of gallic acid in baked oolong tea was presumably originated from the degradation of several phenolic compounds, mainly gallate-type catechins [14,15]. As EGCG is the most abundant phenolic compounds in oolong tea, the detected accumulation of gallic acid in this study seems to be mainly derived from EGCG. According to previous studies, the increase of gallic acid was originated from the decomposition and isomerization of EGCG during the baking processes of tea preparations [14,17]. It seemed that the relative content of gallic acid over EGCG might also be a suitable index for the measurement of baking intensity of oolong teas. Unfortunately, it has been known that the contents of catechins were inversely correlated to the degree of fermentation in the preparation of oolong teas [27,28]. As oolong teas are partially fermented in the preparatory process, the commercially available oolong teas are usually prepared with different degrees of fermentation according to the favorite taste of customers. In this aspect, the contents of EGCG in different oolong teas fluctuate significantly, and thus are not suitable to be used for the measurement of baking intensity of oolong teas unless the original fresh oolong teas are available as the initial controls for their following periodic baking processes.

During the fermentation process in the tea preparation, catechins are activated by endogenous oxidative enzymes, polyphenol oxidase and peroxidase in the cells of tea leaves and tend to dimerize or polymerize to form larger molecules, such as theaflavins, thearubigins and tannins [29,30]. Thus, the contents of catechins are successively reduced in proportion to the degree of fermentation. In contrast with catechins, 5-galloylquinic acid seems to be an inadequate substrate for oxidative enzymes in the tea fermentation process. Hence, the content of 5-galloylquinic acid was found to be unaltered while the content of EGCG was largely reduced in the highly fermented oolong tea analyzed in this study (Fig. 7). In this point of view, the relative content of gallic acid over 5-galloylquinic acid may be more appropriate than the relative content of gallic acid over EGCG to serve as an index for the measurement of baking intensity of oolong teas without knowing the degree of fermentation.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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