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ORIGINAL ARTICLE

Location affects fatty acid composition in *Camellia sinensis* cv Tieguanyin fresh leaves

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Abstract Tieguanyin tea is a typical representative of oolong tea in China, and is famous for its orchid-like aroma. Fatty acids are one of the important precursors for aroma production. However, fatty acid contents and compositions in Tieguanyin largely remain undefined. In this study, we quantified the fatty acid composition in Tieguanyin and its offspring by gas chromatography–flame ionization detector, and compared the effects of growth sites and harvest time on the leaf fatty acid composition. The results showed that total fatty acid contents in Tieguanyin fresh leaves were higher than its offspring. Growth sites had significant impacts on fatty acid contents. Tieguanyin grown in Anxi County showed higher total fatty acid contents, and higher ratio of total unsaturated fatty acids to total saturated fatty acids. The fresh leaves in the morning showed higher total fatty acid contents compared to samples harvested in the afternoon or evening, suggesting a dynamic fatty acid degradation during day period. During tea processing, unsaturated fatty acids including linolenic acid, linoleic acid and oleic acid ($18{:}1^{\Delta 9c}$) decreased 13.1%, 13.2% and 84.2%, respectively. The ratio of unsaturated fatty acids to saturated fatty acids still was above 300%. We found that Tieguanyin was a typical 18:3 plant, and the higher ratio of unsaturated fatty acids to saturated fatty acids of Tieguanyin grown in Anxi County may contribute to its characteristics aroma.

Keywords Tieguanyin · Oolong tea · Fatty acid · Gas chromatography–flame ionization detector

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Introduction

Tieguanyin tea tree, an elite cultivar for oolong tea production, originates from Anxi county of Fujian province. Later on, it is introduced to other regions including Fuzhou, Fuan, Minqing etc., and grows well in the new habitat. However, the aroma quality of Tieguanyin tea produced from different regions varies considerably, it is speculated that the content and profile of aroma precursors of the fresh leaves might vary with growth sites, and contribute to region-specific aroma quality. Fatty acids are well-known aroma precursors in tea, their content increase with leaf maturation (Ravichandran and Parthiban 2000; Mahanta et al. 2010). Fatty acid contents varied with cultivars, seasons or growth regions (Govindasamy et al. 2011; Okal and Owuor 2012; Okal et al. 2012; Wang et al. 2013). During tea processing, fatty acids are oxidized to form volatile aliphatic components, such as hexanal, trans-hexenal, and methyl jasmonate etc. (Sekiya et al. 1984; Ravichandran and Parthiban 2000; Govindasamy et al. 2011), thus shape the characteristics aroma of made tea (Cai et al. 2014; Lv et al. 2014; Zhu et al. 2015). Since manufacture technology influences the degree of fatty acid degradation, different types of tea showed difference in fatty acid contents and composition. The fatty acid contents in green tea and oolong tea are higher than that of black tea (Wang et al. 2009). Fatty acid contents decreased during green tea processing (Ma 2017), and tea cultivars have significant influence on the fatty acid contents of green tea (Liao and Tong 2008). Aroma deterioration during storage is positively correlated with fatty acid content (Wu 1990). In green tea and black tea, fatty acids are extensively studied for their correlations with cultivar, season, manufacture method, shelf life etc., but fewer research has been reported in oolong tea. To understand the contributions of fatty acids to region- or cultivar-specific aroma characteristics in Tieguanyin oolong tea, Tieguanyin tea tree were grown in four different sites and their fatty acid compositions were compared among different locations. In addition, Tieguanyin and its hybrid offspring (Huangguanyin, Jinguanyin, Jinmudan and Zimeigui) were grown in the same location, and their fatty acid compositions also were measured. We found that Tieguanyin fresh leaves grown in Anxi country showed higher ratio of unsaturated to saturated fatty acids, suggesting that fatty acid-derived aroma components may attribute its region-specific aroma quality.

Materials and methods

Tea samples

Huangguanyin (HGY), Jinguangyin (JGY), Zimeigui (ZMG) and Jinmudan (JMD) were the F_1 hybrids of Tieguanyin (TGY) and Huangdan (HD), and oolong tea made from these cultivars had characteristic flavor and aroma. TGY, HD, JGY, ZMG, HGY and JMD were grown in the tea garden of Tea Research Institute of Fujian Academy of Agricultural Science, and the 5 terminal leaves were harvested for fatty acid analysis. For location study, Tieguanyin were grown in Gande town of Anxi County, Shekou town of Fuan County, Xiazhu town of Minqing County, and Cangshan district of Fuzhou city, and their leaves were harvested by the same plucking standard. The temperature in Anxi, Fuzhou, Mingqing and Fuan is 19.5–21.3 °C, 20.0–5.0 °C, 17.0–27.0 °C and 13.6–19.8 °C respectively, and the humidity is 79.3%, 77.0%, 83.0% and 77.0% respectively. To compare the effects of harvest time on fatty acid contents, Tieguanyin fresh leaves were harvested from Gande town in the morning (6:30–7:30 AM, ML), the noon (11:00–12:00 AM, NL), and the evening (16:30–17:30 PM, EL) of the same day. Traditionally, the fresh leaves were harvested in the noon to make oolong tea (NT). After harvesting the fresh leaves were immediately fixed with liquid nitrogen, then freeze-dried before storage in − 80 °C freezer.

Reagents

Sulfuric acid, methanol, butylated hydroxytoluene, toluene and sodium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), FAME reference standard was purchased from J&K Chemical Ltd (Shanghai, China), and margaric acid was purchase from Sigma-Aldrich (Steinheim, Germany). 37 FAME reference standards (C4–C24) were mixture at 100–200 ng/mL, while FAME reference standards (C16–C18) were individual compounds and the purity was at 98–99%.

Methods

Fatty acid analysis

The fatty acid methyl esters preparation followed the method described by Browse et al. (1986). Dry tea samples were grounded into powder by mortar and pestle. Transfer ~ 40 mg powder into a screw-capped glass tube, add 1 mL of 5% sulfuric acid (v/v), 25 μL of 0.2% butylated hydroxytoluene in methanol (w/v), 300 μL of toluene and 75 μg of internal standard margaric acid, then heat at 90 °C for one and half hour. After cooling down to room temperature, 1 mL of 0.9% sodium chloride and 3 mL of hexane were added, vigorously vortexed for 30 s, and then centrifuged at 1000*g* for 10 min. The upper phase was transferred to a new tube. Repeat the extraction step once and combine the supernatant. The supernatant was dried down under a stream of nitrogen gas, the pellet was dissolved in 650 μL of hexane, then filtered through a 0.22 μm membrane before gas chromatography (MDGC/GCMS-2010, Shimadzu, Kyoto, Japan) analysis. The GC was equipped with a RT-2560 column (0.25 mm × 30 m × 0.25 μm, RESTEK, Bellefonte, USA), a flame ionization detector and mass spectrometer. Helium was used as carrier gas at a flow rate of 1 mL/min, the split ratio was 10:1, the temperature setting for inject port and detector was 240 °C and 220 °C, respectively. The oven temperature started at 150 °C, then increased to 200 °C at a rate of 2 °C/min, and held for 5 min. The fatty acids were identified by FAME reference standard and the method of Wang et al. (2017).

Data processing

All of the chemical analyses were carried out in triplicate, and values were expressed as the mean ± standard error (SE). One-way ANOVA analysis was performed to compare the variance resulting from cultivars, growth sites or harvest time, and two-tailed student's *t* test was used to compare the difference between fresh leaves and raw tea.

Results and discussion

Leaf fatty acid contents in Tieguanyin and its offspring

To investigate whether fatty acids have any contributions to cultivar-specific aroma quality, the leaf fatty acid contents of parent lines (TGY and HD) and four F1 hybrid lines (JGY, ZMG, HGY and JMD) were compared. Seven different fatty acids were identified (Fig. 1, Supplementary Table 1 and Supplementary figure S1-S2), including palmitic acid (16:0), palmitoleic acid ($16:1^{\Delta 9}$), stearic acid (18:0), oleic acid ($18:1^{\Delta 9t}$), oleic acid ($18:1^{\Delta 9c}$), linoleic acid ($18:2^{\Delta 9,12}$) and linolenic acid ($18:3^{\Delta 9,12,15}$). Linolenic acid, linoleic acid and palmitic acid were the most abundant fatty acids, and their contents were over 1.0 mg/g dry weight. Linolenic acid was the highest (15.0 $\pm$ 1.5 mg/g dry weight), indicating that tea tree is 18:3 plant.

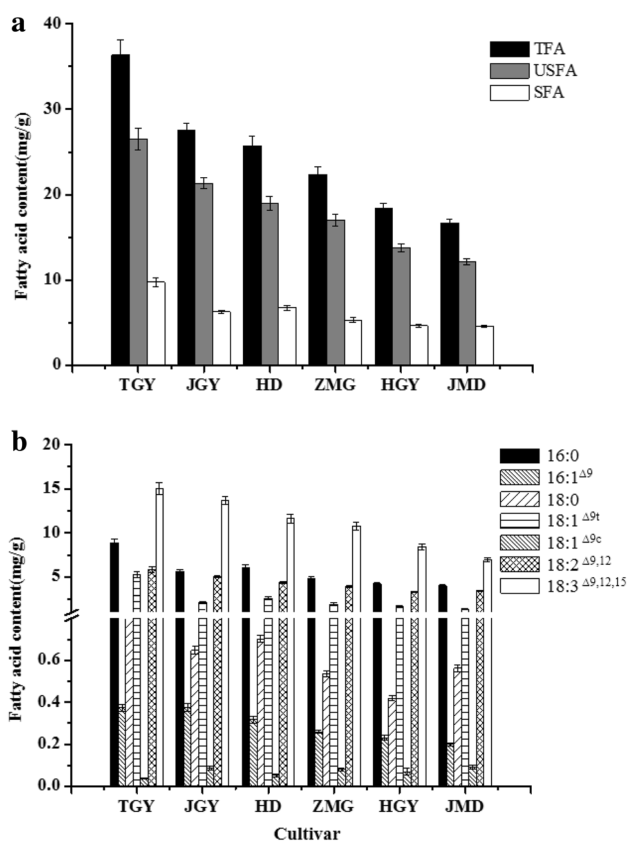

Fig. 1 Fatty acid content and composition in the fresh leaves of different cultivars. **a** JGY, ZMG, HGY and JMD were the offspring of TGY and HD, TFA was total fatty acids in the fresh leaves of different cultivars; USFA was total unsaturated fatty acids in the fresh leaves of different cultivars; USFA was total saturated fatty acids in the fresh leaves of different cultivars. **b** Palmitic acid (16:0), palmitoleic acid ($16:1^{\Delta 9}$), stearic acid (18:0), oleic acid ($18:1^{\Delta 9t}$), oleic acid ($18:1^{\Delta 9c}$), linoleic acid ($18:2^{\Delta 9,12}$) and linolenic acid ($18:3^{\Delta 9,12,15}$) in the fresh leaves of different cultivars were compared

Total fatty acids (TFA) were in the range of 16.6 mg/g to 36.3 mg/g, and there was significant variation among these cultivars (Fig. 1a). TGY showed the highest TFA content, and TFA content of JGY, HD, ZMG, HGY and JMD was 24.1%, 29.1%, 38.5%, 49.4% and 54.1% lower than TGY, respectively. TGY and HD showed a significant difference in TFA as well as individual fatty acid species, only one of their offspring, JGY, showed medium phenotype in TFA content, while other three offspring (ZMG, HGY and JMD) showed even lower TFA than HD.

In consistent with the trend of TFA, USFA content in TGY also was the highest, other varieties were 19.7–66.8% lower than TGY. Most unsaturated fatty acids belonged to C_{18} family, and linolenic acid and linoleic acid contents were highly abundant compared with others. The ratio of USFA to TFA in all the varieties ranged from 72.5 to 77.2%, and JGY showed the highest ratio of USFA to TFA.

The contents of saturated fatty acids showed significant variation among different cultivars (Fig. 1b). TGY had the highest content of total saturated fatty acids (SFA), and all four offspring showed lower SFA content than that of HD. Palmitic acid content was the most abundant SFA. In contrast, stearic acid only accounted for very small portion. Stearic acid content in TGY was 33.1% higher than that of HD, while JGY, ZMG, HGY and JMD were 7.4%, 23.4%, 40.3% and 19.7% lower than that of HD, respectively (Fig. 1a). Moreover, only JGY and ZMG showed USFA to SFA ratio above 300%. Thus, Tieguanyin was rich in unsaturated fatty acids.

Growth sites affected fatty acid contents of Tieguanyin fresh leaves

Okal et al. (2012) reported that the clonal tea grown in different locations showed different levels of fatty acid content. To investigate the location effects on fatty acid contents of Tieguanyin tea, analyze the fatty acids of Tieguanyin fresh leaves collected from four different sites including Anxi, Fuan, Mingqing and Fuzhou. We found that fatty acid contents varied with growth sites (Fig. 2 and Supplementary Table 2). TFA contents varied from 25.6 mg/g to 38.1 mg/g (dry weight), from high to low was Anxi $\approx$ Fuan $>$ Minqing $>$ Fuzhou. TFA, USFA and SFA showed a similar trend except SFA content from Fuan (Fig. 2a). The USFA to TFA ratio were ranged from 72.8 to 79.6%, and Minqing ranked on the top in the ratio of USFA to TFA.

Tieguanyin in Anxi was distinctive for its higher unsaturated fatty acid content, and linolenic acid content was more abundant compared with other regions (Fig. 2b), while the ratio of oleic acid to TFA was less than 1/16. Linoleic acid content was similar to Fuan. Palmitic acid was the major component of saturated fatty acids, and its

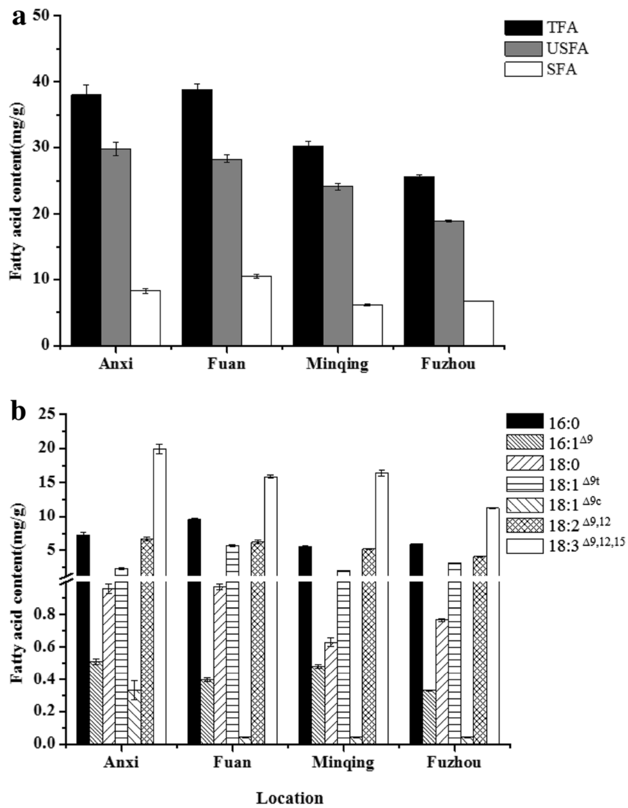

Fig. 2 Fatty acid content and composition in the fresh leaves of different regions (Anxi, Fuan, Minqing and Fuzhou). **a** TFA (total fatty acids), USFA (total unsaturated fatty acids) and SFA (total saturated fatty acids) of fresh leaves were compared between Anxi, Fuan, Minqing and Fuzhou. **b** Palmitic acid (16:0), palmitoleic acid ($16:1^{\Delta 9}$), stearic acid (18:0), oleic acid ($18:1^{\Delta 9t}$), oleic acid ($18:1^{\Delta 9c}$), linoleic acid ($18:2^{\Delta 9,12}$) and linolenic acid ($18:3^{\Delta 9,12,15}$) of fresh leaves in Anxi, Fuan, Minqing and Fuzhou were compared

content in Tieguanyin from Anxi was 23.6% lower than that of Fuan, which resulted in a higher ratio of USFA to SFA (360%) than that of Fuan (270%). It is reported that the ratio of USFA to SFA from tea showed a positive correlation with its aroma quality (Li 1983), and this correlated well with the higher aroma quality of Tieguanyin tea from Anxi compared to other regions.

Harvest time affected fatty acid contents of Tieguanyin fresh leaves

During tea shoot development, (E,E)-2,4-heptadienal, (E,Z)-2,4-heptadienal, (Z)-2-penten-1-ol, hexanal and (Z)-2-heptenal increased (Oyaizu et al. 2002), which attributed to the tea aroma quality improvement. These volatile compounds were derived from fatty acids, and changed with nitrogen fertilizer application and picking interval (Owuor et al. 2013). So far, it remains unclear how fatty acids change daily, and how this affects leaf quality. In order to address these questions, fresh leaves from one site were collected at different time of the same day, and their fatty acid contents were quantified. As shown in Fig. 3 and Supplementary Table 3, fresh leaves plucked at different time of the same day exhibited marked difference in fatty acid contents and composition. ML samples showed higher content of TFA, USFA and SFA, then reduced throughout afternoon (Fig. 3a). These data suggested that portion of leaf fatty acids were degraded during day period. This may explain why tea garden typically shows strong fresh scent notes during a sunny afternoon since green leaf volatiles (leaf aldehyde, (2*E*)-hexenal, leaf alcohol, (3Z)-hexenol, etc.) were derived from fatty acids oxidation (Hatanaka 1996; Ito and Tanaka 2014). Harvest time also significantly affected fatty acid composition of Tieguanyin fresh leaves (Fig. 3b). Different fatty acids showed similar trends with plucking time, including palmitic acid (16:0), palmitoleic acid ($16:1^{\Delta 9}$), stearic acid (18:0), oleic acid ($18:1^{\Delta 9t}$), oleic acid ($18:1^{\Delta 9c}$), linoleic acid ($18:2^{\Delta 9,12}$) and linolenic acid ($18:3^{\Delta 9,12,15}$), and the order from high to low was ML $>$ NL $\approx$ EL. These observations suggested that in the morning fatty acid degradation rate from Tieguanyin fresh

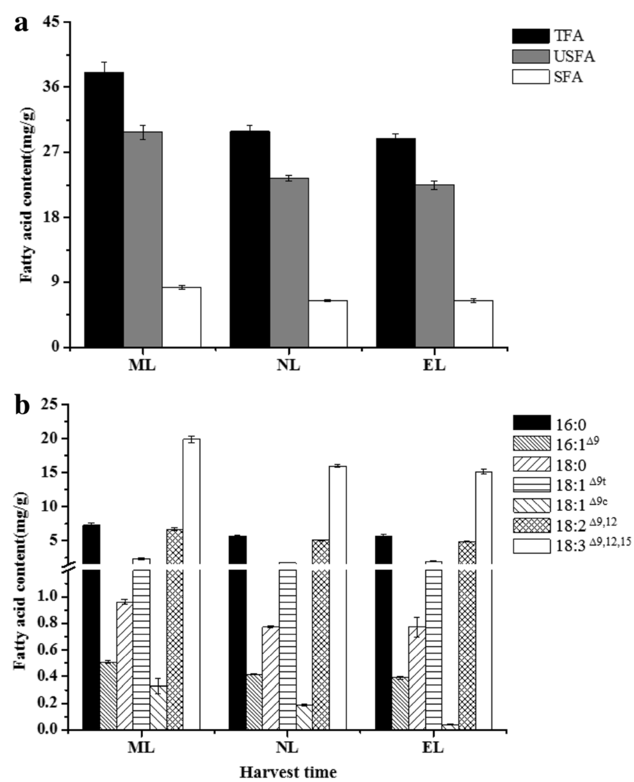

Fig. 3 Fatty acid content and composition in the fresh leaves of different harvest time (in the morning, at noon and in the evening). **a** TFA (total fatty acids), USFA (total unsaturated fatty acids) and SFA (total saturated fatty acids) of fresh leaves were compared between different harvest time. **b** Palmitic acid (16:0), palmitoleic acid ($16:1^{\Delta 9}$), stearic acid (18:0), oleic acid ($18:1^{\Delta 9t}$), oleic acid ($18:1^{\Delta 9c}$), linoleic acid ($18:2^{\Delta 9,12}$) and linolenic acid ($18:3^{\Delta 9,12,15}$) of fresh leaves in different harvest time were compared

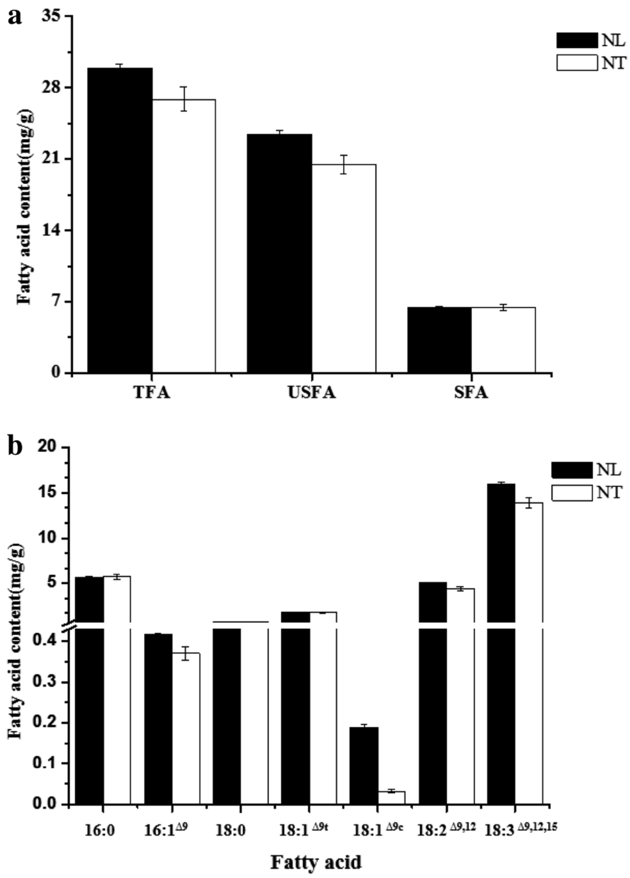

Fig. 4 Fatty acids in the fresh leaves and oolong tea. **a** Oolong tea was made by TGY fresh leaves harvested in the noon; TFA (total fatty acids), USFA (total unsaturated fatty acids) and SFA (total saturated fatty acids) of fresh leaves were compared with oolong tea; * was the difference of fatty acids at the level of $P < 0.05$. **b** Palmitic acid (16:0), palmitoleic acid ($16{:}1^{\Delta 9}$), stearic acid (18:0), oleic acid ($18{:}1^{\Delta 9t}$), oleic acid ($18{:}1^{\Delta 9c}$), linoleic acid ($18{:}2^{\Delta 9,12}$) and linolenic acid ($18{:}3^{\Delta 9,12,15}$) of fresh leaves were compared with oolong tea; * and ** were the difference of fatty acids at the level of $P < 0.05$ and $P < 0.01$ respectively

leaf was faster than that of synthesis. Browse et al. (1981) reported that there was a diurnal fluctuation in the proportions of 18C unsaturated fatty acids in the lipids of developing spinach leaves, where the concentration of oleic acid increased during the day and declined subsequently at night.

Fatty acid degradation during Tieguanyin tea processing

About 85% fatty acids were liberated during black tea processing, linolenic acid, linoleic acid and palmitic acid underwent further degradation (Lin et al. 2014). During green tea making, the fatty acid content also declined (Ma 2017). We found that the fatty acid content and composition from oolong tea changed significantly comparing with that of fresh leaves. TFA and USFA content of oolong tea were 10.1% and 12.8% lower than that of fresh leaves, while SFA content unchanged (Fig. 4a and Supplementary Table 4). However, the ratio of USFA to SFA in oolong tea was still above 300%.

Among the five unsaturated fatty acids detected from fresh leaves and oolong tea, linolenic acid, linoleic acid and oleic acid ($18{:}1^{\Delta 9c}$) showed significant reduction comparing with that of fresh leaves, while palmitoleic acid ($16{:}1^{\Delta 9}$) and oleic acid ($18{:}1^{\Delta 9t}$) kept unchanged (Fig. 4b). Linolenic acid content was decreased to 13.9 ± 0.6 mg/g (dry weight) after tea processing, still 2.2 times and 13.3 times higher than linoleic acid and oleic acid ($18{:}1^{\Delta 9c}$), respectively.

Conclusion

In this study we found that Tieguanyin fresh leaves contained higher levels of fatty acids than its offspring (JGY, HGY, ZMG and JMD). The USFA to SFA ratio from Tieguanyin fresh leaves was over 300% when grown in Anxi County. During day time, the fatty acid contents of fresh leaves in the noon and evening were lower than that in the morning. During oolong tea processing, linolenic acid, linoleic acid and oleic acid ($18{:}1^{\Delta 9c}$) decreased significantly. However, the USFA to SFA ratio in oolong tea still was above 300%.

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