



## ARTICLE

# Leaf Wettability Difference Among Tea Leaf Ages and Analysis Based on Microscopic Surface Features

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Received: 26 April 2022 Accepted: 06 June 2022

## ABSTRACT

The wettability of leaf surface, commonly represented by contact angle (CA), affects various physiological and physical processes. The present study aims to better understand the wettability of tea leaves and elucidate its influence on the energy barrier of the droplet condensation process. The CA values of different leaf ages (young, mature and old) of five famous tea cultivars (*Maolu*, *longjing* 43, *Huangjinya*, *Zhongcha* 108 and *Anji Baicha*) were measured via the sessile drop method, and the micro-morphology of two cultivars leaves (*Maolu*, *Zhongcha* 108) was investigated by a 3D super depth-of-field digital microscope. Specifically, two radically distinctive types of CA trends were observed, one was the decreased firstly and then increased slightly with the increase of leaf age, while the other stayed constant. The valley depth or maximum height ( $R_z$ ) of *Maolu* leaf surface increased with the leaf age while the  $R_z$  of *Zhongcha* 108 leaf remained unchanged by comparing the microscopic features. The *Maolu* mature leaf CA decline attributed to the young leaf was hydrophilic ( $\theta < 90^\circ$ ), and it was considered that surface structures like folds and pits on old leaf played a crucial role in making CA increased. Small deviation in CA can lead to significant error in calculation of the contact angle function of energy barrier in phase change. It will have great significant for simulating and better understanding the formation of frost on tea leaves.

## KEYWORDS

Wettability; contact angle; tea; surface; energy barrier

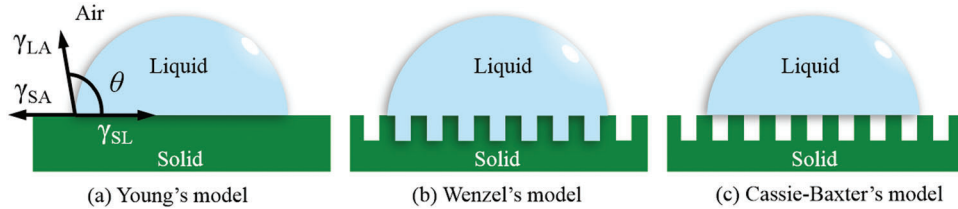
## 1 Introduction

Tea (*Camellia sinensis* [L.] O. Kuntze) is one of the high-value cash crops in hilly areas of China, and tea products has become the most consumed beverage worldwide [1]. Frost happening frequently in late spring causes serious damages on tea leaves, resulting in huge quality reduction and economic losses [2,3]. Generally, before frost forms on the surface, there will be visible condensation droplets on it [4–6]. After the liquid droplet changes to a solid state, frost crystals and branches grow on the frozen droplets [7]. Wettability of surface directly affects the droplet condensation and solidification in frosting [8]. In addition, leaf wettability is also an important characteristic related to canopy water retention, gas exchange [9] and bacterial transmission [10], which affects the quality of tea.

Wettability is most often assessed on the basis of contact angle (CA) value, measured between a horizontal solid surface and fluid interface [11]. The CA is usually a constant quantity when a droplet



statically spread on a horizontal surface [12]. The classical Young's model [13] was employed to describe this ideal wetting phenomenon (Fig. 1a). However, absolutely smooth leaf surfaces are nonexistent in nature, which most typical example is 'lotus effect' [14]. Wenzel [15] considered the bottom liquid of the droplet infiltrate the micro-structure and modified Young's model by taking the surface roughness into consideration (Fig. 1b). Afterwards, Cassie et al. [16] proposed a new theoretical model by considering the proportion of different components on the surface (Fig. 1c). In this state, composite interface underneath the liquid was formed by air pockets and play a key role to help us to understand the wetting behavior.



**Figure 1:** Three classic wetting models, where  $\gamma_{SL}$ ,  $\gamma_{SA}$  and  $\gamma_{LA}$  are the interfacial tensions of solid-liquid, solid-air, liquid-air, respectively;  $\theta$  is contact angle (CA)

Generally, the wetting traits of a solid surface can be classified into three types. First one is the superhydrophobic surface, when the CA of droplet is over  $160^\circ$ , the surface is considered to be water repellency. The second type is a hydrophobic surface with a CA above  $90^\circ$ . The last one is the hydrophilic surface which can be easily wetted by water with CA lower than  $90^\circ$  [17]. Leaf CA is usually measured by the sessile droplet method using a contact angle meter even though it is not an artificial solid surface [18]. Droplet will roll off and without water spread if leaf surface has hydrophobic property. Conversely, the leaf surface shows hydrophilic property when droplets spread or adhere to it [19].

The wetting behavior of plant surfaces is much more complicated with limited researches than that of abiotic rigid surfaces. Hanba et al. [20] investigated the influence of leaf wettability on photosynthesis in bean and pea. The  $\text{CO}_2$  assimilation of bean leaves (hydrophilic) differs from that of pea leaves (hydrophobic) and will be reduced after subjecting them to water mist. Papierowska et al. [21] pointed that leaf wettability has effect on droplet splashing phenomenon, which also influence diseases spreading [22]. The largest dimensionless diameter of droplet on leaves decreased with hydrophobic property increased. Tea leaf, Zhu et al. [23] analyzed the influence of wettability on surfactant droplet maximum retention. Several factors affecting the leaf wettability have also taken into account and discussed. Holder [24] measured the CA of 36 species of tree leaves and found that leaf wettability varied greatly between regions. Zhu et al. [25] observed that rice leaf CA increased with growth and there were no distinct difference between cultivars. Papierowska et al. [18] indicated that the wettability of potato leaf was closely related to trichome types and density. Moreover, wax mass and structure also influence leaf wettability [26].

Interestingly, there are some intrinsically different conclusions about the effect of leaf age on leaf wettability. Holm oak leaf CA decrease with the increase of leaf age whether on the adaxial or abaxial surface [27]. This indicates that the CA of young holm oak leaf is maximum. However, not all plant leaf CA values are distributed so regularly. For example, abaxial surface CA of European beech young leaf at top canopy is minimal in October [28]. In addition, young leaf of rice is more easily wetted compared to leaves with other ages, which also means that young leaf CA is minimal [25]. It can be seen that leaf age has different effects on leaf wettability of different plants. Meanwhile, the effect on the leaf age of tea leaf is still poorly studied.

Therefore, the study aims to quantitatively describe the influence of tea leaf ages on its wettability by measuring CA, and a microscopic analysis was conducted to interpret the differences in wettability. It can provide a theoretical basis for simulating and better understanding the formation of frost on tea leaf.

## 2 Materials and Methods

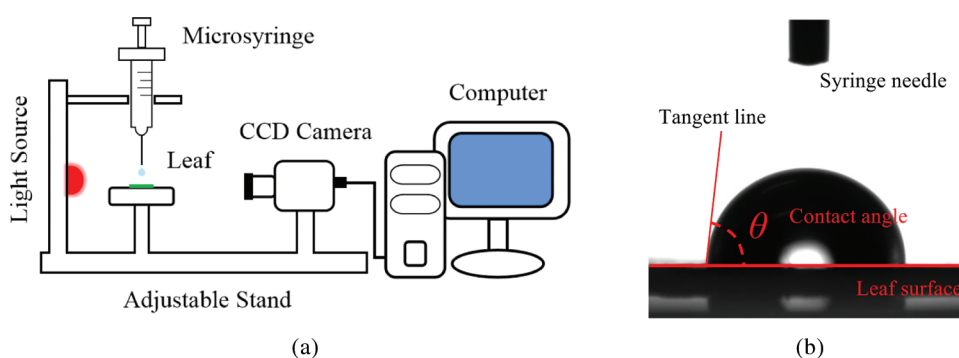
### 2.1 Leaf Material and Preparation

Five cultivars of famous tea in Jiangsu Province, China were selected for this research. These cultivars were *Maolu*, *longjing 43*, *Anji Baicha*, *Zhongcha 108* and *Huangjinya*. Six fresh leaves of different age (young, mature and old) of each cultivar were collected and transported to the laboratory in a cool chamber on December 02, 2021. Those leaves were rinsed using deionized water to remove surface contaminants and then dried at ambient temperature. Both left and right sides of the midrib were cut off and 12 test samples of each cultivar were made. To obtain a flat surface, each part of abaxial surface was carefully fixed to glass slide with double sided adhesive tape.

In this study, young leaves were from the second or third fully unfolded leaf below the terminal bud; leaves that were 5 to 7 leaves away from the bud were considered mature leaves and those having many years of age on the bottom canopy were selected as old leaves. All leaves were guaranteed to be intact without any injury.

### 2.2 Contact Angle (CA) Measurements

The wettability of tea leaves of various ages of each cultivar was obtained by measuring a distilled water droplet CA with a device (CAM 200, KSV, Finland, Fig. 2a). It was connected to a computer with built-in software for data processing. The measurement was conducted in the laboratory via the sessile drop method, given the ambient temperature of 293 K and relative humidity of 60%. The critical volume of the pure water droplet for measuring the leaf CA was about 10  $\mu\text{L}$  [9]. The droplet with the volume of 10  $\mu\text{L}$  was generated through a syringe, shown in Fig. 2b, and photographed roughly 20 s after dropping. The CA of the adaxial leaf surface was measured 10 times and the average value was taken as the final result.



**Figure 2:** Experimental device and measuring method. (a) Schematic diagram of contact angle meter; (b) water droplet of 10  $\mu\text{L}$  was measured via the sessile drop method

An analysis of variance (ANOVA) was performed on the differences in the measured data using Origin. Tukey test was used for mean comparison and significance levels were taken as 0.05 [9]. There was a significant difference between the two sets of data if the  $P$ -value was less than 0.05.

### 2.3 Microscopic Leaf Observation

A 3D super depth-of-field digital microscope (VHX-200, KEYENCE, Japan) was used for observing the surface structure of the examined leaves by photographing at the same magnification ( $\times 500$ ). The microscope

allowed easy observation of the three-dimensional leaf surface morphology as well as measurement of the desired parameters. It was found that the center light source at the lens made the images clearer. Three photographs of each sample were chosen from a three times repeated experiment.

## 2.4 Calculation of Energy Barrier

Water vapor will condense into liquid cores if it touches a cooler surface. It can spontaneously condense vapor molecules to a droplet when the liquid core was larger than a certain radius. The Gibbs free energy of the system caused by the formation of a critical liquid core is called the energy barrier. According to the phase transformation dynamics, the energy barrier was considered as:

$$\Delta G_c = \frac{V}{\phi} \Delta g + S_{LA} \cdot \sigma_{LA} + S_{SL} \cdot \sigma_{SL} - S_{SA} \cdot \sigma_{SA} \quad (1)$$

where  $\phi$  is a single molecule volume;  $\Delta g$  is the energy change of a single molecule. Assuming that the droplets condensed on a leaf surface are spheroidal as shown in Fig. 1a, then its volume was:

$$V = \frac{1}{3} \pi R^3 (2 - 3 \cos \theta + \cos^3 \theta) \quad (2)$$

The contact areas between the different phases will be:

$$S_{LA} = 2\pi R^2 (1 - \cos \theta) \quad (3)$$

$$S_{SA} = S_{SL} = \pi R^2 \sin^2 \theta \quad (4)$$

where  $S_{LA}$  and  $S_{SL}$  are the contact area of liquid-air and solid-liquid, respectively;  $S_{SA}$  is the reduced area of solid-air;  $\theta$  is CA and  $R$  is the sphere radius .

So the  $\Delta G_c$  can be derived as [29]:

$$\Delta G_c = \left( \frac{4\pi R^3}{3\phi} \Delta g + 4\pi R^2 \sigma_{LA} \right) f(\theta) \quad (5)$$

In particular,  $f(\theta)$  is the contact angle function:

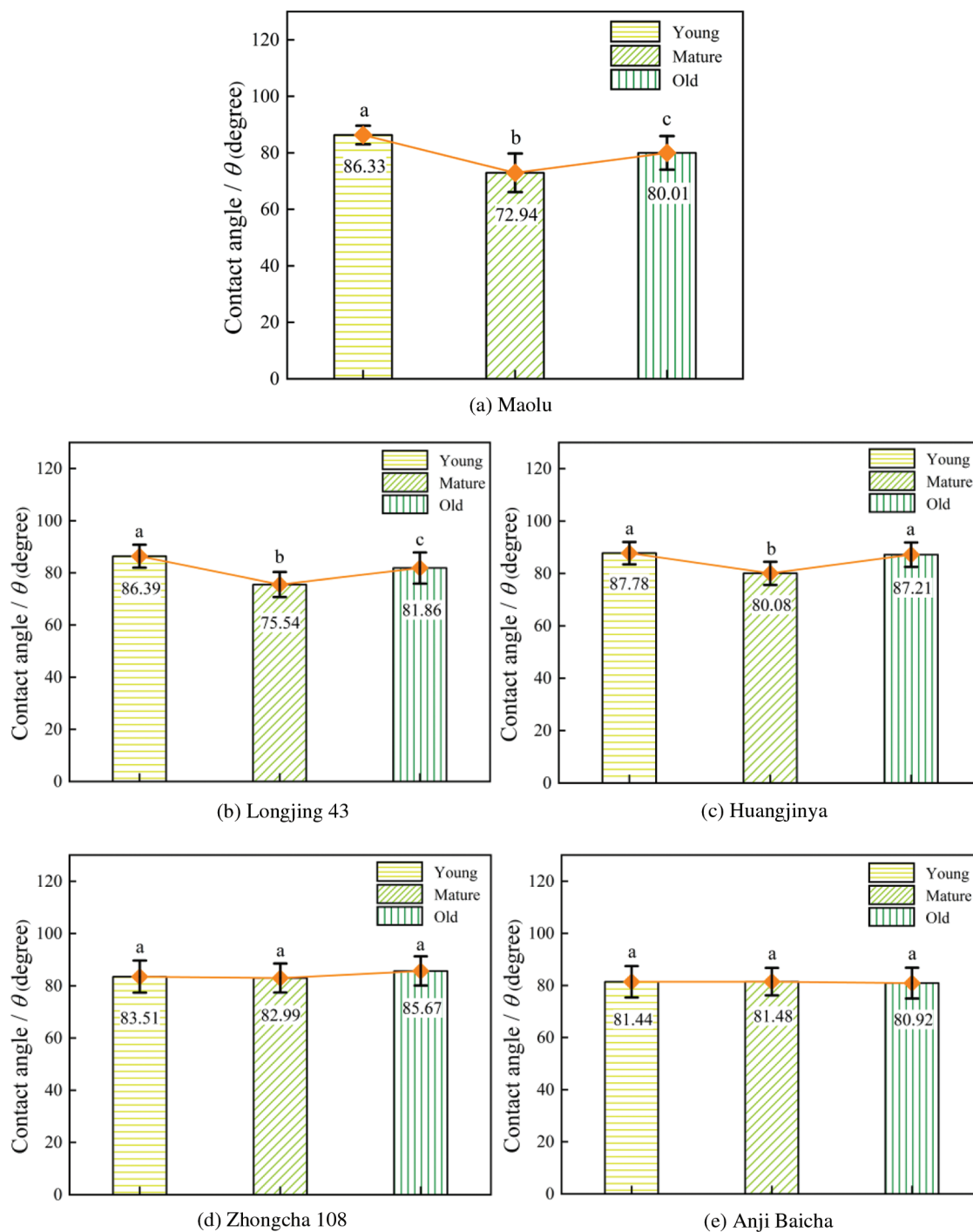
$$f(\theta) = \frac{1}{4} \cos^3 \theta - \frac{3}{4} \cos \theta + \frac{1}{2} \quad (6)$$

## 3 Results and Discussion

### 3.1 Contact Angle Measurements

The different letters above the columns of Fig. 3 represent that the  $P$ -value between any two data sets was lower than 0.05 and there was a significant difference. *Maolu* leaves CA values were significantly different (Fig. 3a). The CA values showed the obvious trend that firstly decreased and then increased and had no varied gradient with the leaf age. The young leaf CA was  $86.33^\circ$  which was  $13.39^\circ$  and  $6.32^\circ$  higher than that on mature and old leaf CA, respectively. Meanwhile, the CA of old leaf increased  $7.07^\circ$  vs. mature leaf CA.

A similar distribution of leaf CA values was also observed for *longjing 43* and *Huangjinya* leaves. The CA of young leaf was maximum while the mature leaf CA firstly decreased and then old leaf CA increased slightly, as shown in Figs. 3b and 3c. However, *Zhongcha 108* and *Anji Baicha* leaves CA values kept nearly constant with the various leaf ages (Figs. 3d and 3e).



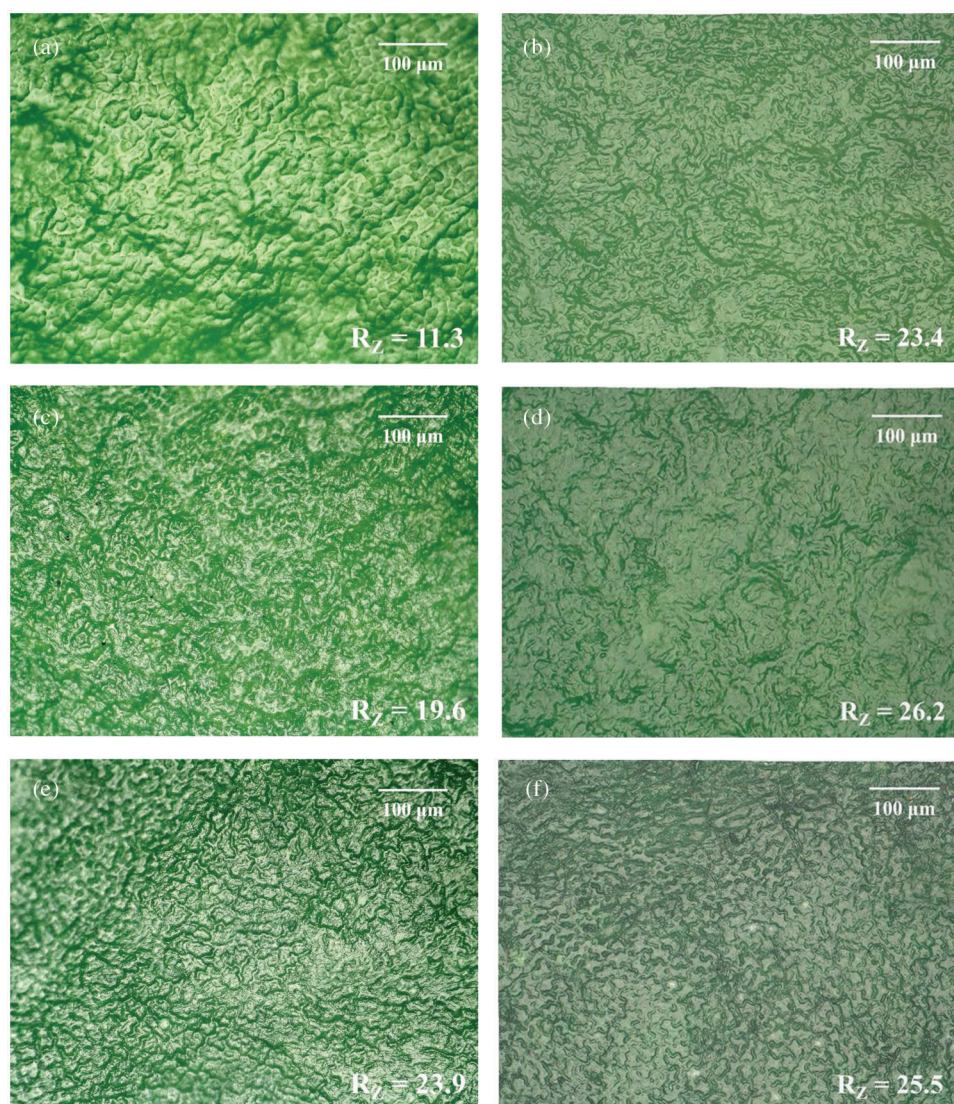
**Figure 3:** The leaf CA of 5 cultivars

Leaves of different ages had CA values less than  $90^\circ$  and were considered hydrophilic surface according to accepted classification criteria [17–19]. The CA of *Huangjinya* was the largest among young leaves, but it was still below  $90^\circ$ . For mature leaves, it was found that the CA of *Huangjinya* decreased compared with that of the young leaf. Mature leaf CA of *Zhongcha 108* was the largest, while the CA of *Maolu* was the smallest

which was around  $70^\circ$ . Remarkably, *Huangjinya* old leaf CA was the largest value ( $87.21^\circ$ ). The maximum and minimum average CAs were on *Huangjinya* and *Maolu* with values of  $85.02^\circ$  and  $79.76^\circ$ , respectively.

### 3.2 Microscopic Features of Leaves

The  $\theta$  of *Maolu*, *longjing 43* and *Huangjinya* had the trend of first decreased and then increased. However, the CA of *Maolu*'s leaf had the most obvious differences among leaf ages, while those of *Zhongcha 108* and *Anji Baicha* leaves of different ages were similar. *Maolu* and *Zhongcha 108* leaf surfaces had different CA trends and were selected for microscopic observation. The 3D morphology images of different leaves of *Maolu* and *Zhongcha 108* are shown in Fig. 4.



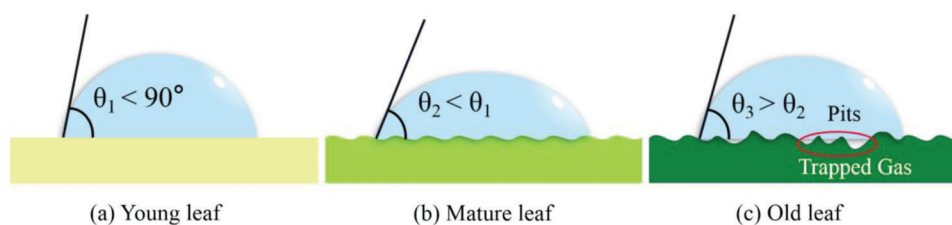
**Figure 4:** 3D images of leaf surface morphology. (a), (c) and (e) are young, mature and old leaves of *Maolu*; (b), (d) and (f) are young, mature and old leaves of *Zhongcha 108*, respectively, with magnification of 500 times

Fig. 4a shows that the young leaf surface of *Maolu* was tender, and the valley depth or maximum height ( $R_Z$ ) was only  $11.3 \mu\text{m}$ . On the contrary, the young leaf of *Zhongcha 108* had obvious surface folds with a higher  $R_Z$  of  $23.4 \mu\text{m}$  (Fig. 4b). Figs. 4c and 4d show the morphology of mature leaves. The color of *Maolu* mature leaf was darker and the  $R_Z$  increased to  $19.6 \mu\text{m}$ . The surface morphology of *Zhongcha 108* mature leaf was similar to the young leaf,  $R_Z$  had a slight increase to  $26.2 \mu\text{m}$ . In Fig. 4e, the surface color of *Maolu* old leaf turned to black green and the gaps were clear. The  $R_Z$  of old leaf of *Zhongcha 108* was  $25.5 \mu\text{m}$  which had almost no change compared with that on young and mature leaves.

The schematic diagrams of *Maolu* leaf and CA variation are in Fig. 5. The young leaf was relatively smooth as the morphological observation results shown which can be simplified as an ideal flat surface (Fig. 5a). The classic Young's model was used to describe this ideal wetness phenomenon:

$$\gamma_{SV} = \gamma_{LV} \cos \theta + \gamma_{SL} \quad (7)$$

where  $\gamma_{SL}$ ,  $\gamma_{SA}$  and  $\gamma_{LV}$  are solid and liquid, solid and air, liquid and air interface tensions.



**Figure 5:** Schematic diagrams of tea CA change (not to scale). The young leaf was considered as an ideal flat surface and CA was  $\theta_1$ ; folds were increased on mature leaf and CA was  $\theta_2$  which was lower than  $\theta_1$ ; folds and pits of the old leaves were further deepened and CA was  $\theta_3$  which was larger than  $\theta_2$

Revisiting the 3D morphology and structure results in Fig. 4c, obvious folds appeared on the surface with the growth of leaves, and the  $R_Z$  of mature leaf increased. Inspired by classical wetting theories, the CA decreased can be reasonably interpreted by the Wenzel's model:

$$\cos \theta_W = r \cos \theta \quad (8)$$

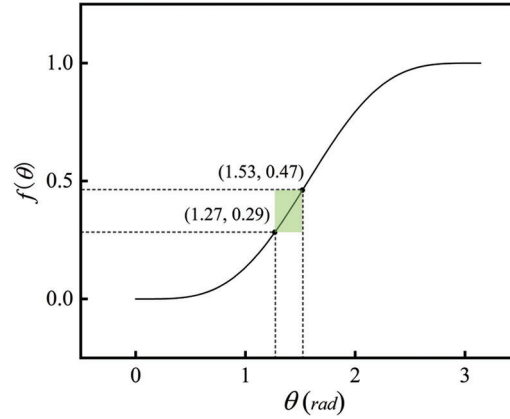
where  $\theta_W$  and  $\theta$  are the Wenzel's CA and the Young's CA, respectively,  $r$  is the ratio of the actual area of droplet contact to the projected area on the horizontal plane, known as the roughness factor. A hydrophilic surface ( $\theta < 90^\circ$ ) will become more hydrophilic with the increase of roughness according to this model. It can better explain the CA of *Maolu's* mature leaf reduction compared to its young leaf.

In nature, surface structure of plant leaves can significantly influence the CA. For instance, the homogeneous distribution of papillae structures on the superhydrophobic surface of lotus leaf [30], effectively support the bottom of the droplet. Anisotropic groove shape on the surface also make superhydrophobic the reed [31] and ginkgo leaves [32]. Machining similar surface structures is one method of increasing surface CA in industry. Those microscopic structures on leaves allow liquid to impregnate or partially penetrate into them. What's more, the eggbeater shaped structure of salvinia leaf can form a thin air layer that prevents water from contacting the leaf surface further [33]. Similarly, the bounce of droplets falling at low velocity on other stationary droplet is also thought to be generated by the air between the surfaces [34].

A slight increase in CA of *Maolu* old leaves was observed instead of a decrease. We considered that the main cause of this result was that microscopic surface features like folds and pits developed further on the old leaf, and let droplets more difficult to intrude into the gaps, as shown in Fig. 5c. The effect of surface defects distribution and size on leaf CA needs further study.

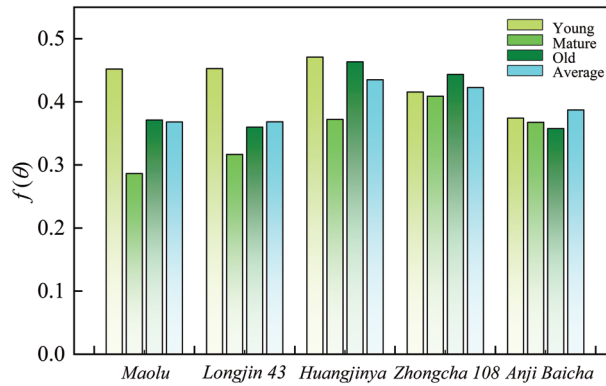
### 3.3 Influence of CA on Energy Barrier

The  $f(\theta)$  value was determined completely by the CA according to Eq. (6). Fig. 6 shows that the variation of  $f(\theta)$  increases from 0 to 1 with the CA changing from 0 to  $\pi$ . It can be seen that the  $f(\theta)$  value varies greatly with CA within a certain range. Small changes in leaf CA may have a major effect on the calculation of energy barrier.



**Figure 6:** The variation of  $f(\theta)$ . The radian of tea CA ranges from 1.27 to 1.53 in this study which determined a significant rising stage of the function

The  $f(\theta)$  of tea leaves must be calculated when droplet condensation occurs on the surface. As the Fig. 7 shows, the  $f(\theta)$  value of *Maolu* mature leaf ( $CA = 72.94^\circ$ ) was 0.29 while that of young leaf ( $CA = 86.33^\circ$ ) was almost 55.2% higher. The  $f(\theta)$  values of various cultivars at the same age were also significantly different. For instance, the mature leaf  $f(\theta)$  value of *Maolu* was 42.8% lower than that of *Zhongcha 108* ( $CA = 82.99^\circ$ ). The average CA of one cultivar was the arithmetic mean of the CAs at three leaf ages, which might be reasonable for some cultivars. However, the  $f(\theta)$  value calculated from average CA will have a large error if leaf CA varies greatly with age. The  $f(\theta)$  value calculated from *Maolu* average CA was 18.58% lower than that when the young leaf CA was used. Therefore, it is necessary to first investigate whether the leaf CA is influenced by leaf age before studying the phase transformation process on tea leaves.



**Figure 7:**  $f(\theta)$  values corresponding to different CA

#### 4 Conclusions

The CA values of tea leaf surfaces of different cultivars and various leaf ages were measured in this research which were all less than  $90^\circ$ , and belonged to hydrophilic surfaces. But two variation trends of CA were observed with increasing of leaf age. One was the trend of firstly descend and then slightly ascend with the leaf age. There was a gradual increase in the  $R_z$  of the leaf surface, the hydrophilic surface contributed to the CA decreases firstly. For the old tea leaf surface, there were obvious defects on it and these small folds or pits structures hinder the spreading of droplets which made the CA increases slightly. Another trend in CA is that it remained essentially constant with leaf age. The accurate CA of the target leaf should be measured when calculating the energy barrier. The CA error of a dozen degrees can cause the deviation in  $f(\theta)$  larger than 50%.

**Authorship:** The authors confirm contribution to the paper as follows: study conception and design: Qingmin Pan, Yongguang Hu; data collection: Yongzong Lu; analysis and interpretation of results: Qingmin Pan, Liang Xue, Yongguang Hu; draft manuscript preparation: Qingmin Pan, Yongzong Lu. All authors reviewed the results and approved the final version of the manuscript.

**Funding Statement:** This research was funded by the Key Research and Development Program of Jiangsu Province (BE2021340), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (21KJB210019), the Jiangsu Postdoctoral Science Foundation (2021K614C), the Key Laboratory of Modern Agricultural Equipment and Technology, Jiangsu University (MAET202119), the Project of Postgraduate Innovation of Jiangsu Province (KYCX22\_3683) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD-2018-87).

**Conflicts of Interest:** The authors declare that they have no conflicts of interest to report regarding the present study.

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