



## Pu-erh tea tasting in Yunnan, China: Correlation of drinkers' perceptions to phytochemistry

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### ABSTRACT

**Aim of the study:** Pu-erh (or pu'er) tea tasting is a social practice that emphasizes shared sensory experience, wellbeing, and alertness. The present study examines how variable production and preparation practices of pu-erh tea affect drinkers' perceptions, phytochemical profiles, and anti-oxidant activity.

**Materials and methods:** One hundred semi-structured interviews were conducted in Yunnan Province to understand the cultural and environmental context of pu-erh tea tasting. The *gong fu cha dao* ('way of tea' with 'effort,' 'work,' or 'skill') method of brewing tea through multiple infusions was employed to evaluate green and black pu-erh samples from smallholder agro-forests and terrace plantations. Ranking interviews, High Performance Liquid Chromatography (HPLC), and the 1-1-diphenyl-2-picrylhydrazyl (DPPH) assay were conducted to characterize color and taste profiles, Total Catechin Content (TCC), Total Methylxanthine Content (TMC), and free radical scavenging capacity (IC<sub>50</sub>).

**Results:** Significant variation was found among pu-erh samples based on: (1) agro-ecosystem mode of production by TCC ( $P < 0.0001$ ) and TMC ( $P < 0.0265$ ), (2) processing method for TCC ( $P < 0.0001$ ), TMC ( $P < 0.0027$ ), and free radical scavenging capacity ( $P < 0.0001$ ), (3) infusion sequence for TMC ( $P < 0.0013$ ), (4) taste rankings for TCC ( $P < 0.0001$ ), TMC ( $P < 0.0001$ ), and IC<sub>50</sub> ( $P < 0.0059$ ) and, (5) color rankings for TMC ( $P < 0.0009$ ) and IC<sub>50</sub> ( $P < 0.0001$ ). Samples rated as bitter and bitter-sweet contained the greatest TCC and free radical scavenging capacity.

**Conclusions:** This research demonstrated that production environment, processing methods, and infusion sequence in preparing tea are related to the phytochemical profile, free radical scavenging activity, and flavor of tea. Findings contribute to the ethnomedical literature by supporting previous studies that have hypothesized that the taste of plants, particularly bitterness, may guide societies in the search for medicinal plants and beneficial phytochemicals.

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### 1. Introduction

Pu-erh (or pu'er) tea tasting is a social practice that emphasizes shared sensory experience, wellbeing, and alertness. Pu-erh refers to processed leaves and buds from the broad-leaf variety of the tea plant (*Camellia sinensis* var. *assamica* (L.) O. Kuntze; Theaceae) native to the Upper Mekong River Region of China's Yunnan Province (Ming and Zhang, 1996) and contiguous parts

of China, Laos, Vietnam, Myanmar, and India. Yunnan's forests and smallholder agro-ecosystems are the center of diversity of tea (Chen and Pei, 2003; Chen et al., 2005), including 12 wild relatives (section *Thea* of *Camellia*; Ming and Zhang, 1996; Long et al., 2003) and hundreds of cultivars and landraces (Xiao and Li, 2002). The diverse environments and cultural practices involved in tea production by Yunnan's multiple socio-linguistic groups contribute to tea with wide-ranging sensory profiles. Tea, as a botanical with multiple markets, functions, and cultural uses, appeals to varied tastes (Purdue, 2008). The desire for varied sensory experiences among pu-erh tea drinkers provides feedback that helps to maintain and enhance the diversity associated with tea production. Pu-erh tea tasting has developed prominence over the past

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two decades in response to expanded market integration of pu-erh tea from Yunnan. Consequently, historical tea production and consumption practices are being revitalized and adapted for the contemporary socio-economic context.

The present study examines how variable production and preparation practices of pu-erh tea affect drinkers' perceptions, phytochemical profiles, and anti-oxidant activity. The *gong fu cha dao* ('way of tea' with 'effort,' 'work,' or 'skill') method of brewing tea through multiple infusions was employed to evaluate green, aged, and black pu-erh samples from smallholder agro-forests (or *gushu cha yuan*/'old tree tea gardens') and terrace plantations. The overall aims of this research are to understand the role of drinkers' perceptions in recognizing production variables and phytochemical levels of tea and, to inform studies on human selection of medicinal plants. Semi-structured and ranking interviews, High Performance Liquid Chromatography (HPLC), and the 1-1-diphenyl-2-picrylhydrazyl (DPPH) assay were employed to characterize the experience of pu-erh tea tasting. The following introduction is based on literature review and the authors' field research during the design of this study.

### 1.1. Customary use of pu-erh tea

Numerous socio-linguistic groups in Yunnan Province, including the Bulang (Blang), Wa, Akha (Hani), Lahu, Yao, Hmong (Miao), Jinuo, De'ang, Dai, and Han, have produced pu-erh for centuries. They consume tea as a medicine, tonic, beverage, and food for energy and wellbeing. Pu-erh is also consumed as a social beverage and for continuation of cultural traditions. Some of the health-related claims attributed to pu-erh include strengthening the immune system, balancing the body's hot and cold levels, detoxifying blood, treating rheumatism and stones, remedying headaches, and reducing swelling and soft tissue. Pu-erh has several health-related claims associated to mental wellbeing including invigorating the mind and relieving stress. Additionally, pu-erh is valued for providing nutrition, aiding digestion, and preventing obesity.

A few socio-linguistic groups in Yunnan prepare pu-erh as a food. For example, upland Bulang communities ferment pu-erh in underground pits for several weeks to years and eat the leaves as an accompaniment or salad. The Bulang also eat fresh tea with *nanmi* (a condiment made of several spices) and elder Bulang women chew tea leaves with a mixture of betel nut (*Areca catechu* L.; *Arecaceae*), lime, and other plants. Fresh and fermented tea leaves are particularly eaten during celebrations such as the annual harvest and nature worship ceremony in tea agro-forests. The Jinuo roast tea leaves that are mixed with spices and wrapped in banana leaves.

### 1.2. Bioactive constituents and taste attributes

Catechins, a group of polyphenolic flavan-3-ol monomers and their gallate derivatives, are the primary compounds responsible for the health-related claims of unfermented tea (Zhen, 2002). The methylxanthine compound caffeine contributes to tea's stimulant properties. These secondary metabolites are defense compounds that provide plants with resistance to pathogens and predators (Ames et al., 1990), oxidative stress, and protection from other environmental variables. Their amounts vary depending on genetic and environmental conditions (Chung et al., 1998). The major catechins in tea are (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epigallocatechin-3-gallate (EGCG). Catechins undergo oxidation during the processing from green to black tea to form the oxidized high molecular components bisflavanol, theaflavin, and thearubigin (Zhen, 2002). Statins, a class of fermentation-derived compounds, are produced during the microbial fermentation process of certain post-fermented tea, such as black pu-erh (Jeng et al.,

2007). Numerous studies have reported tea catechins to demonstrate a range of cellular mechanisms that have anti-oxidative, anti-inflammatory, neuro-protective, anti-cancer, anti-microbial, and anti-atherosclerotic activities (Clement, 2009). Despite the extensive research on tea, epidemiological and clinical studies are inconclusive. However, clinical studies have substantiated green tea extracts as the source of the only botanical prescription drug approved by the United States Food and Drug Administration (FDA) (Chen et al., 2008).

A combination of catechin, methylxanthine, carbohydrate, and amino acid compounds contribute to the distinctive taste and color of tea (Drewnowski and Gomez-Carneros, 2000; Scharbert et al., 2004). Catechins have been documented as bitter, astringent, and bitter with a sweet aftertaste (Yamanishi, 1990). Theobromine is described as bitter and metallic (Rouseff, 1990), caffeine is bitter (Hall et al., 1975), and the amino acids are sweet and sour (Nelson et al., 2002). Catechins are colorless or light-colored compounds and their oxidative products, the theaflavins and thearubigins, possess the dark brown and orange-red color attributes that are characteristic of black teas (Liang et al., 2003).

### 1.3. Variability of pu-erh production

The Pu, an ancestral people of the Bulang, Wa and De'ang, are considered the first cultivators of the tea plant (Huang, 2005). Tea cultivation in southwestern China is estimated to date over 1700 years (Xiao and Li, 2002). Tea in Yunnan grows in forests, agro-forests, mixed crop fields, and terrace plantations. Forest tea includes tea trees that are wild, sparsely planted in forests, or trees that were cultivated and have become feral. Tea agro-forests (known as 'ancient teagardens' in Yunnan) are forest areas (0.5–3.0 ha.) thinned for tea cultivation or swidden areas where plant regeneration is fostered. They are most often managed by smallholder upland communities in Yunnan, including the Bulang, Wa, Akha, Lahu, Yao, Hmong, Jinuo, and De'ang. The multi-storied vegetative structure of agro-forests provides soil fertility and pests and disease control (Jose, 2009). Mixed crop tea plots are created in tea agro-forests by replacing associated woody plants with rice or other crop production and by pruning tea trees to increase irradiance and promote crop growth. Agro-forests and mixed crop plots are examples of low intensive agro-ecosystems. In contrast, terrace tea (*taidi cha*/'tableland tea') plantations are agricultural-intensive monoculture systems. They are large fields most often managed for uniformity, high-yield, and efficiency where tea plants are cultivated in compact rows and are pruned to waist-high shrubs. This production mode usually relies on chemical fertilizer and pesticide input.

Pu-erh is most often processed as a compressed tea of various brick, cake, log, nest, and gourd shapes. Following harvest, tea leaves are processed as a type of loose green tea (*san cha* or 'scattered tea'), which is the raw material for pressed green pu-erh (*sheng bing* or 'raw cake'), aged pressed green pu-erh (*lao bing* or 'old cake'), and pressed black pu-erh (*shu bing* or 'cooked cake'). The processing of loose green pu-erh starts with withering and heat fixing leaves by pan-frying them on a wok to lower the moisture content and deactivate oxidative enzymes, e.g. polyphenol oxidase, catalase, peroxidase and ascorbic acid oxidase (Zhen, 2002). Pan-fried leaves are rolled by hand or by mechanical grinders in order to disrupt the cell walls to remove additional moisture and shape the plant material. Kneaded leaves are spread out, typically on bamboo mats, and sun dried to prevent spoiling and to capture the 'taste of the sun' (*tai yang wei*). These processing steps are similar to that of other green teas that function to prevent oxidation of phytochemical constituents (Zhen, 2002), however the deactivation of enzymes is not as complete for pu-erh. Consequently, pu-erh has a distinct oxidizing process and develops a smooth taste with age.

Pressed green pu-erh is prepared by steaming loose green pu-erh and compressing the supple leaves into desired shapes using bamboo or stone molds. The compressed tea is then sun dried. Historically, pressed green pu-erh was a main commodity on the caravan trade routes from Yunnan and Sichuan provinces to Tibet, Nepal, India, and Burma. These trade paths are now collectively known as the Southwest Silk Road (*Xi'nian Sichouzhilu*) or Tea-Horse Road (*Chama Dao*; Yang, 2004). Sections of the Southern Silk Road are estimated to date over 1000 years (Yang, 2004). Tea oxidized and fermented during the trade journey as it interacted with moisture and temperature fluctuations, and its flavor transformed from bitter to mellow.

Today, connoisseurs store pressed green pu-erh in clay jars, bamboo wrapping and baskets, caves, and underground pits in attempt to optimally age tea and transform its flavor. The characteristics of aged pu-erh, can artificially be imparted by a microbial food-processing technology (*hou fa jiao*, 'post-fermentation,' 'cooking,' or 'ripening') that was developed for pu-erh in the 1970s. The resultant black pu-erh involves oxidizing and heap-fermenting leaves for several hours to days following the rolling process of loose green pu-erh. Temperatures and moisture are often adjusted for controlled post-fermentation and leaves may intentionally be inoculated with selected microorganisms such as *Aspergillus* sp. (*Trichocomaceae*) (Ku et al., 2010). The oxidized and fermented leaves are then steamed, compressed, and dried. Black pu-erh is categorized as a post-fermented tea. The microorganisms in black pu-erh oxidize polyphenol compounds more completely than the enzymatic oxidation of other black teas (Xie et al., 2009), and create fermentation-derived compounds known as statins.

#### 1.4. Gong fu cha dao tea preparation

The *gong fu cha dao* ('way of tea' with 'effort,' 'work,' or 'skill') method of brewing tea is currently being revitalized and adapted in Yunnan in response to the recent expansion of the pu-erh market. *Gong fu cha dao* developed in eastern China and draws from earlier tea preparation methods documented in Lu Yu's *Cha Jing* ('Classic of Tea,' written around 760–780 CE), considered the first monograph of tea (Lu, 1974). The *gong fu cha dao* method is based on multiple infusions of briefly steeping leaves in a lidded bowl or in an unglazed clay teapot (Fig. 1). The number of infusions, brewing duration, leaf amount, and water temperature used in preparing pu-erh varies with production and processing variables of tea and drinker preferences. Some pu-erh drinkers interviewed for this study during preliminary research claim that brewing tea through brief and multiple infusions functions to optimally release its aroma, taste, color, and physiological properties, thus providing for a 'balanced composition' that brings out nuanced essences.

A notable component of pu-erh tea tasting is for drinkers to compare and communicate their sensory perceptions of each infusion and to discern characteristics of how and where the tea was grown and processed. This practice is considered to keep the mind and senses vigilant through focus and communication. The first infusion prepared during the pu-erh tea preparation, and occasionally the second, is poured out in order to open the dried leaves and release their sensory properties for the following infusions. In some Tibetan communities of Yunnan surveyed by this study's authors, pu-erh tea infusions are distributed in a hierarchical order. The most elder or senior drinkers are offered the first serving and the youngest or least senior drinkers receive the last infusions.

#### 1.5. Theoretical framework

Humans have an intrinsic distaste for bitter substances, and the rejection and detoxification of these substances is thought to be an adaptation for survival (Glendenning, 1994; Hladik and

Simmen, 1996). Johns (1990) hypothesized that despite the association of bitter substances with toxicity, some societies may have evolved mechanisms for selecting plants with bitter, astringent, and unpalatable properties for nutritional and pharmacological sources, sensory variety, and cultural identity. The earliest written mentions of tea are found in its documentation as a medicine and gruel in the 3rd and 4th centuries (Ceresa, 1996). Early documentation of tea generally describe its taste was bitter (Zhen, 2002). Some tea producers and consumers continue to select bitter tea for its potential health benefits. Research on perceptions of the organoleptic properties of plants and their associated medicinal properties is important towards understanding the process of experimentation, discovery, and selection in ethnomedical systems (Casagrande, 2000). The taste of plants, particularly bitterness, may guide societies in the search for medicinal plants and beneficial phytochemicals (Etkin, 1988; Moerman, 1998).

## 2. Materials and methods

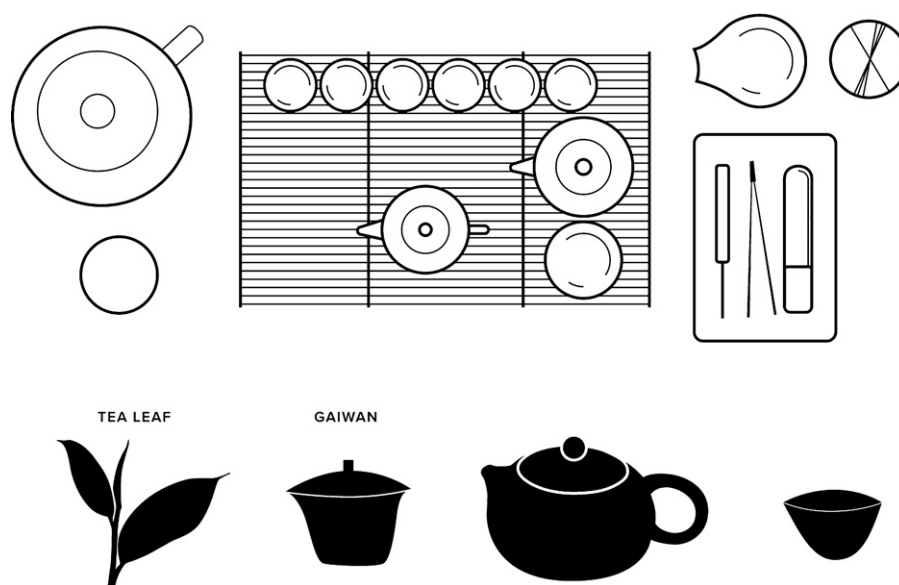
### 2.1. Interviews on perceptions of pu-erh

One hundred semi-structured interviews were conducted with tea producers, traders, connoisseurs, and masters in Yunnan Province, China. All informants gave prior informed consent to volunteer in this study. Interviews with tea producers were carried out in upland Bulang, Ahka, Yao, Lahu, and Yi ethnic communities in Xishuangbanna, Honghe, and Lincang prefectures of southern Yunnan. Interviews with tea masters, connoisseurs, and traders were administered in tea markets, tearooms, tea processing factories in urban and rural areas in Yunnan. A preparation protocol was developed based on observation of 30 pu-erh tea tastings that recorded infusion time, water and tea quantity, and brewing temperature. This protocol was employed for preparing samples for analysis of color, taste, phytochemical, and anti-oxidant profiles.

Five informants were identified as key experts based on semi-structured interviews with tea masters who host pu-erh tastings in teahouses in Yunnan. During regularly scheduled pu-erh tastings, key experts were requested to compare perceptions of production, medicinal properties, taste, and color between tea infusions of 10 pu-erh samples. A rating scale methodology was employed to quantify taste and color based on a similar scale and respondent sample size as Thorngate and Noble (1995). Key experts were asked to rate the strength of taste for each infusion on a 5-point category scale, with one being the lowest taste strength and five the highest taste strength. Similarly, informants were asked to rate the color intensity for each infusion based on a 4-point category scale, with one being the lowest intensity of color (lightest) and four the highest (darkest). Informants were also requested to share their perceptions of production variables of each sample and indicate samples perceived to have the greatest potential health-benefiting properties and stimulant properties.

### 2.2. Plant material

The 100 tea infusions assessed for this study were derived from 10 pu-erh samples from southwestern Yunnan. Five of the pu-erh samples (or 50 of the tea infusions) were from agro-forests and the other five samples (or 50 of the tea infusions) were from terrace plantations. Samples from each mode of production were processed as green pu-erh (or 'raw'/sheng; 3 samples), aged green pu-erh (4 samples), and black pu-erh (or 'cooked'/shu; 3 samples). The green and black pu-erh samples were analyzed within 6 months of their production and the aged green pu-erh samples were all over 10 years from their date of production. Voucher specimens of tea (*Camellia sinensis* var. *assamica* (L.) O. Kuntze; Theaceae) were



**Fig. 1.** Tea setting for the *gong fu cha dao* ('way of tea' with 'effort,' 'work,' or 'skill') method of brewing tea that is based on multiple infusions of briefly steeping tea leaves in a lidded bowl (*gai wan* or 'lid bowl') or in an unglazed clay teapot.

collected from agro-forests and deposited at the Kunming Institute of Botany, Chinese Academy of Sciences.

### 2.3. Chemicals and reagents

One hundred tea infusions were prepared in the lab with spring water (Poland Spring, Wilkes Barre, PA). HPLC-grade acetonitrile (purchased from J.T. Baker, Phillipsburg, NJ), trifluoroacetic acid (Fisher Scientific, Fair Lawn, NJ) and distilled water (Milli-Q system, Millipore Laboratory, Bedford, MA) were used for High Performance Liquid Chromatography to separate and identify study compounds. Gallic acid, ascorbic acid, sodium carbonate, 1-1-diphenyl-2-picrylhydrazyl (Sigma Chemical Co., St. Louis, MO, USA), and reagent-grade ethanol (Fisher Scientific, Fair Lawn, NJ) were used as solvents and controls to measure free radical scavenging capacity. Pure standards of gallic acid (GA), (+)-catechin (C), caffeine (CAF), (–)-epigallocatechin 3-gallate (EGCG) and (–)-gallocatechin (GC) were purchased from ChromaDex (Santa Ana, CA). Standards of (–)-epicatechin 3-gallate (ECG), (–)-epigallocatechin (EGC), and (–)-catechin 3-gallate (CG) were purchased from Fisher Scientific (Pittsburgh, PA) and standards of theobromine (TB), theophylline (TP), (–)-gallocatechin 3-gallate (GCG) and (–)-epicatechin (EC) were bought from Sigma Chemical Co. (St. Louis, MO).

### 2.4. Sample extraction

Samples were prepared in the laboratory using a *gong fu cha dao* protocol of multiple infusions developed during participant observation of 30 pu-erh tea tastings. Ten infusions were prepared in a gaiwan using 8 g of each of the 10 samples (100 infusions total). The first infusion of each sample was prepared by steeping 8 g of dry leaves in 80 ml of spring water at 95 °C for 30 s (Fig. 1). The leaves steeped for the first infusion were re-steeped for the second through 10th infusions using 80 ml of spring water at 95 °C for 20 s. The resultant extracts were filtered under vacuum with No. 5 Whatman paper, frozen at –20 °C, lyophilized to obtain dried extracts and weighed. One gram of each extract was re-suspended in 10 ml of water. Aliquots (1.5 ml) of the filtrate were centrifuged at 15,000 rpm for 15 min and the supernatant was passed through a 0.45 μm nylon membrane filter prior to HPLC and DPPH analysis.

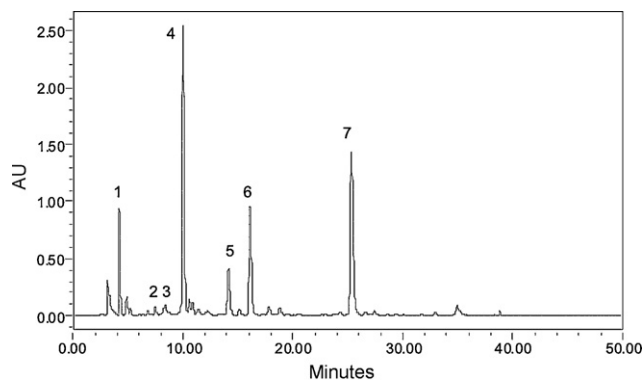
### 2.5. HPLC for quantification of phenolic and methylxanthine compounds

HPLC (High Performance Liquid Chromatography) quantification of samples was performed using a Waters 2695 HPLC (Milford, MA) module equipped with a 996 photodiode array detector (PDA). Sample compounds and standards were separated on a Synergi Fusion, 4 μm, 250 mm × 4.6 mm ID, C-18 reversed-phase column (Phenomenex, Torrance, CA). Column temperature was maintained at 30 °C and sample temperature was kept at 4 °C. A gradient system was used for the mobile phase elution consisting of 0.05% (v/v) trifluoroacetic acid in distilled water (Solvent A) and in acetonitrile (Solvent B) at a flow rate of 1.0 ml/min for a duration of 35 min. The following gradient profile was used, as previously described by Dalluge et al. (1998): 0–25 min, 12–21% B; 25–30 min, 21–25% B. The column was reconditioned between sample injections by flushing it with 100% Solvent B for 10 min and then re-equilibrating the column for 5 min to starting conditions. Forty microliters (μL) of each of the 100 infusions was run in single injections for HPLC analysis. The UV-vis spectra were recorded from 254 to 400 nm and peaks representing the 12 catechin and methylxanthine compounds investigated were detected at 280 nm. Peaks of the study compounds were identified based on their characteristic absorbance spectra and retention time (Fig. 2).

The HPLC method employed for this study was adapted from a method previously validated by some of this study's authors (Unachukwu et al., 2010) with respect to selectivity, linearity, detection, quantification limits, and precision. The limit of detection (LOD) and the limit of quantification (LOQ) of seven standards, GA, C, CAF, EGCG, ECG, TP, and TB were found to be in the ranges of 0.05–1 and 0.1–5 μg/ml, respectively (Unachukwu et al., 2010). The sample extraction for the present study was modified from the previously validated extraction method (Unachukwu et al., 2010) according to the traditional *gong fu cha dao* method of brewing pu-erh tea through multiple infusions.

### 2.6. DPPH for analysis of free radical scavenging capacity

Free radical scavenging capacity was assessed using the 1-1-diphenyl-2-picrylhydrazyl (DPPH) assay as a measure of antioxidant activity as described in Saito et al. (2007). Forty infusions



**Fig. 2.** Chromatogram of an aged green pu-erh tea sample analyzed using Synergi Fusion C<sub>18</sub> reverse phase HPLC column 4  $\mu$ m, 250 mm  $\times$  4.6 mm ID by gradient elution during method development of this study. Detection was carried out with UV at A<sub>280</sub>. Peak identification are as follows: (1) gallic acid (GA), (2) (–)-epigallocatechin (EGC), (3) (+)-catechin (C), (4) caffeine (CAF), (5) (–)-epigallocatechin 3-gallate (EGCG), (6) (–)-gallo catechin 3-gallate (GCG), and (7) (–)-catechin 3-gallate (CG).

of pu-erh representing each processing method from agro-forests were selected for DPPH analysis. Gallic acid (0.015625–0.25 mg/ml) and ascorbic acid (0.03125–0.5 mg/ml) were used as positive controls. Fifty  $\mu$ L of extracts and control dilutions was mixed with 150  $\mu$ L of 400  $\mu$ M DPPH solution; each mixture was prepared in quadruplicates of five concentrations. The mixtures were incubated for 30 min at 37 °C. Absorbance values were measured at 517 nm using a Softmax Pro 3.0 microplate reader (Molecular Devices, Sunnyvale, CA). Radical scavenging capacity of each sample was calculated as the percentage of DPPH free radicals inhibited by the sample in comparison to radical inhibition in the negative water control using the following formula:

$$\frac{ABS_{\text{negative control}} - ABS_{\text{sample}}}{ABS_{\text{negative control}}} \times 100.$$

Values obtained were plotted against concentration ( $\mu$ g/ml) of sample dilutions. Free radical scavenging capacity was expressed as IC<sub>50</sub> values (concentration of sample required to scavenge 50% of DPPH radicals; the lower the IC<sub>50</sub> value, the higher the anti-oxidant activity).

### 2.7. Statistical analysis

Results were analyzed statistically using JMP 7.0 software (SAS) to determine mean values of quantified masses of compounds analyzed by HPLC and IC<sub>50</sub> values assessed by DPPH. Total Catechin Content (TCC; expressed as mg/g dry tea) was determined by the addition of the individual amounts of catechins quantified from HPLC results (EGCG, ECG, EGC, GCG, CG, GC, EC, and C). Similarly, Total Methylxanthine Content (TMC; expressed as mg/g dry tea) was determined by the addition of the individual methylxanthine compounds quantified from HPLC results (CAF, TB, and TP). Aggregate values of analyte quantities for each sample were calculated by the addition of the 10 infusions brewed per sample. The relationship of production, processing, color, and taste variables to TCC, TMC, and IC<sub>50</sub> were determined using Analysis of Variance of pooled means with a significance level of  $\alpha = 0.05\%$ . Tukey–Kramer HSD was performed to compare all mean pairs. Differences with  $P \leq 0.05$  values were considered significant.

## 3. Results

### 3.1. Perceptions of pu-erh tea tasting

Informants used multiple taste and physiological descriptors to capture the sensory experience of pu-erh tea tasting. The most

prevalent taste terms used by informants included bitter (58%), bitter with a sweet aftertaste (57%), and sweet (54%). Table 1 lists the taste terms used by informants to describe pu-erh. A total of 73% of informants said that bitter or bitter-sweet taste indicates medicinal properties of tea. Informants perceived tea with a bitter and bitter-sweet taste to be grown in a forest, 'ecological,' 'natural' or 'healthy cultivation environment.' Urban informants referred to bitter pu-erh from forest and agro-forests as 'bitter tea' (*ku cha*), 'ecological tea' or 'natural tea' (*sheng tai cha*), 'big tree tea' (*da shu cha*), or 'ancient tree tea'. Informants recognized numerous factors associated with production environment to impact the taste of pu-erh, including harvest time, season, location, plant height and age, cultivar or landrace, altitude, fog, slope, temperature, canopy cover, soil, rain fall, and humidity. The experience of the pu-erh tea tasting is additionally described based on physiological sensations (uplifting, mouth-watering, throat drying, calming), fragrance (sweet, floral, citrus), color (golden, amber, red), and clarity (clear, dusty, cloudy).

All key experts rated green pu-erh samples from agro-forests with the strongest taste and described these samples as either bitter or bitter with a sweet aftertaste. Key experts were able to identify between tea samples from agro-forests and terrace plantations because of the more bitter taste of the former. Green pu-erh samples were also perceived to contain the greatest medicinal and stimulant properties. Key experts rated black pu-erh with the mildest taste and the least caffeine content. They described this tea as smooth and perceived it to contain the least medicinal and stimulant properties. Aggregated informant responses indicated that infusions 4–7 had the strongest taste and infusions 1, 9 and 10 were rated with the mildest taste.

### 3.2. Variation of phytochemical constituents and free radical scavenging capacity

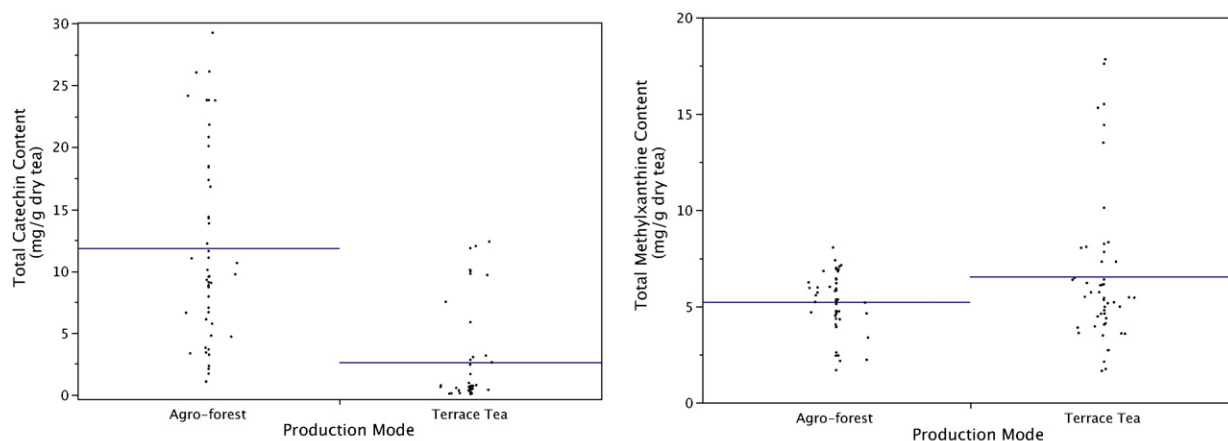
Total Catechin Content (TCC) of aggregate infusions of individual pu-erh samples ranged from 1.35 to 238.52 mg/g dry tea with a mean of 71.90 mg/g dry tea. ECG had the highest mean value of all catechins quantified, and was found in greatest quantity in a green pu-erh sample harvested from an agro-forest (91.71 mg/g dry tea for aggregate infusions). Total Methylxanthine Content (TMC) of aggregate infusions of individual samples ranged from 43.83 to 98.27 mg/g dry tea with a mean of 58.67 mg/g dry tea. Caffeine had the highest mean value of all methylxanthine compounds quantified and was most present in a sample of aged green pu-erh from a terrace plantation (94.60 mg/g dry tea for aggregate infusions).

Results show significant variation among samples based on mode of production for TCC ( $P < 0.0001$ ) and TMC ( $P < 0.0265$ ) (Fig. 3). Samples from agro-forests had higher mean TCC than samples from terrace plantations, and samples from terrace plantations had higher mean TMC. Significant variation was also found among samples based on processing method for TCC ( $P < 0.0001$ ),

**Table 1**

Most prevalent taste terms mentioned during interviews in Yunnan to describe the pu-erh tea tasting experience.

| Taste term                     | Frequency (%) | Taste term | Frequency (%) |
|--------------------------------|---------------|------------|---------------|
| Bitter                         | 58            | Smooth     | 26            |
| Bitter with a sweet aftertaste | 57            | Mellow     | 23            |
| Sweet                          | 54            | Full       | 22            |
| Sour                           | 39            | Fishy      | 22            |
| Salty                          | 34            | Moldy      | 21            |
| Astringent                     | 33            | Earthy     | 21            |
| Acidic                         | 33            | Metallic   | 19            |
| Floral                         | 31            | Acrid      | 17            |
| Smoky                          | 34            | Vegetal    | 12            |
| Fruity                         | 27            | Burnt      | 2             |



**Fig. 3.** Total Catechin Content (TCC; sum of EGCG, ECG, EGC, GCG, CG, GC, EC, and C) and Total Methylxanthine Content (TMC; sum of CAF, TB, and TP) of individual pu-erh infusions by mode of production system: agro-forest versus terrace tea plantation. Horizontal lines represent the mean TCC and TMC values.

TMC ( $P < 0.0027$ ), and free radical scavenging capacity ( $P < 0.0001$ ) (Fig. 4). Mean TCC based on processing method was found to be in the order green pu-erh > aged green pu-erh > black pu-erh, and mean TMC was found as aged green pu-erh > green pu-erh > black pu-erh. Fig. 5 shows mean analytes per infusion by processing method. Mean free radical scavenging capacity was found in the order green pu-erh > aged green pu-erh > black pu-erh (Fig. 4; Table 2 shows the individual  $IC_{50}$  values and standard deviation of the 40 infusions assessed in quadruplicates). Means comparisons of TCC for all pairs of processing methods showed significant variation ( $P < 0.007$ ), while means comparisons of TMC for all pairs of processing methods showed significant variation between green pu-erh and aged green pu-erh ( $P < 0.0205$ ), and between aged green pu-erh and black pu-erh ( $P < 0.0051$ ). Insignificant variation was found for TMC between green and black pu-erh ( $P < 0.8941$ ). In addition, mean comparisons of  $IC_{50}$  values for all pairs of processing methods found significant variation between green and black pu-erh ( $P < 0.0001$ ), and between green and aged green pu-erh ( $P < 0.0143$ ). Insignificant variation was found for  $IC_{50}$  between aged green and black pu-erh ( $P < 0.1784$ ).

Aggregate data of all samples found the fourth infusion brewed through the *gong fu cha dao* method of multiple infusions contained

the highest mean TCC, the third infusion contained the highest mean TMC, and the eighth infusion contained the highest mean free radical scavenging capacity (Fig. 6). However, comparison of infusion sequence only found significant variation for TMC ( $P < 0.0013$ ), and insignificant variation for TCC ( $P < 0.9949$ ) and  $IC_{50}$  ( $P < 0.4298$ ). Comparison of all means of the different infusion sequences found insignificant variation between TCC, TMC, and  $IC_{50}$ .

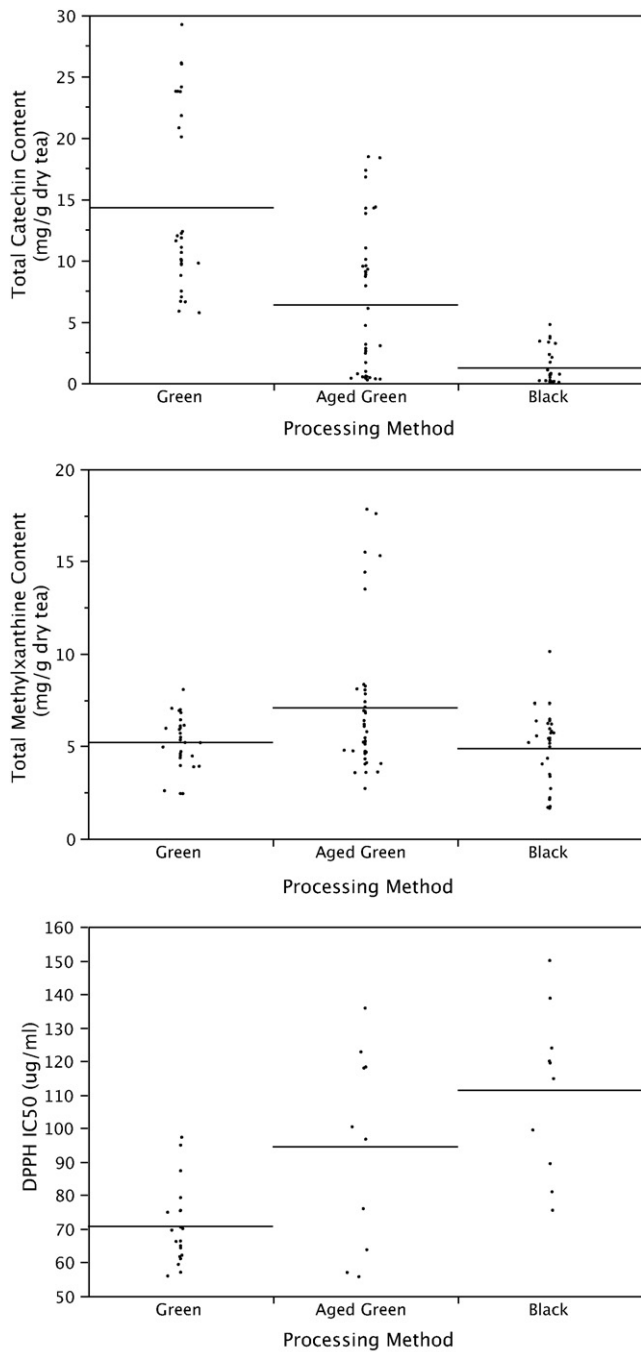
### 3.3. Relationship of phytochemical profiles and perceptions of pu-erh tea tasting

Infusions rated with the strongest taste (level 5 on the ranking scale) had the highest mean TCC, TMC, and free radical scavenging capacity (indicated by lowest  $IC_{50}$ ) (Fig. 7). These infusions were prepared from green pu-erh samples and were perceived by key informants to contain the greatest medicinal properties of samples evaluated. The samples rated with the weakest taste were found to contain the lowest TCC and free radical scavenging capacity. These infusions were prepared from black pu-erh and were perceived to have the least medicinal properties. No correlation was found between informants' perceptions of stimulant properties and TMC. Infusions rated level 3 on the color scale had the highest TMC and

**Table 2**

Free radical scavenging capacity of pu-erh samples from agro-forests measured by  $IC_{50}$  values.  $IC_{50}$  is the concentration of sample required to scavenge 50% of DPPH radicals (the lower the  $IC_{50}$  value, the higher is the free radical scavenging capacity).

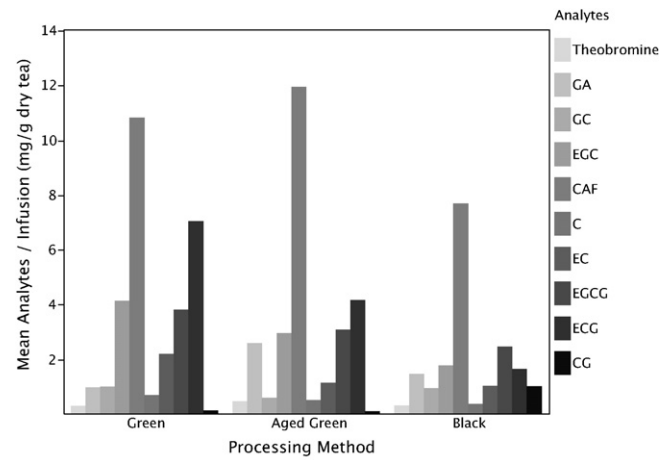
| Infusion # | $IC_{50}$ value        | Standard deviation | $IC_{50}$ value   | Standard deviation |
|------------|------------------------|--------------------|-------------------|--------------------|
|            | Green pu-erh (loose)   |                    | Aged green pu-erh |                    |
| 1          | 64.31                  | 1.27               | 96.71             | 9.88               |
| 2          | 64.94                  | 1.26               | 118.23            | 23.74              |
| 3          | 66.36                  | 1.79               | 100.41            | 5.54               |
| 4          | 59.41                  | 1.44               | 135.73            | 5.02               |
| 5          | 70.08                  | 5.46               | 117.89            | 5.26               |
| 6          | 69.62                  | 0.97               | 122.71            | 10.09              |
| 7          | 55.95                  | 0.77               | 76.01             | 13.21              |
| 8          | 57.07                  | 1.33               | 63.79             | 10.57              |
| 9          | 61.10                  | 0.90               | 55.75             | 4.42               |
| 10         | 61.72                  | 3.47               | 57.01             | 2.39               |
|            | Green pu-erh (pressed) |                    | Black pu-erh      |                    |
| 1          | 97.28                  | 8.25               | 149.94            | 8.88               |
| 2          | 74.92                  | 14.46              | 138.72            | 20.86              |
| 3          | 87.30                  | 7.79               | 119.98            | 5.36               |
| 4          | 70.52                  | 1.96               | 114.74            | 5.48               |
| 5          | 66.23                  | 2.03               | 123.86            | 8.26               |
| 6          | 75.49                  | 2.09               | 119.40            | 10.56              |
| 7          | 79.31                  | 3.28               | 99.46             | 6.40               |
| 8          | 62.14                  | 2.70               | 89.43             | 24.36              |
| 9          | 94.93                  | 9.13               | 75.52             | 15.39              |
| 10         | 75.37                  | 2.37               | 81.04             | 5.46               |



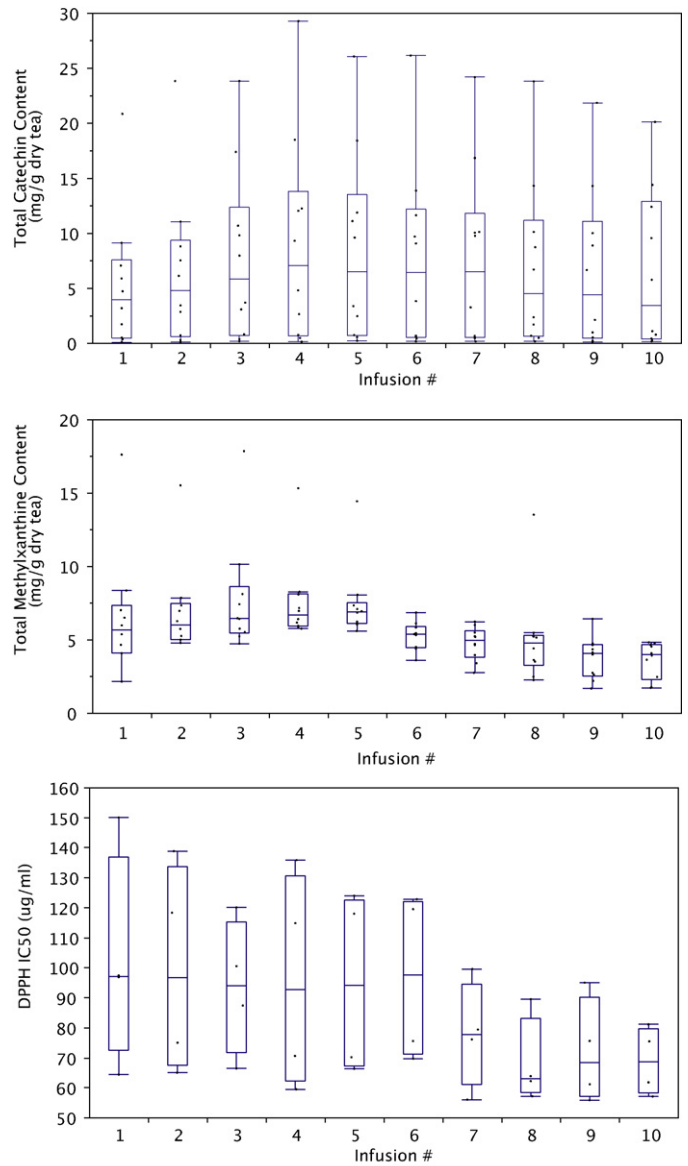
**Fig. 4.** TCC (Total Catechin Content), TMC (Total Methylxanthine Content), and IC<sub>50</sub> values (measure of free radical scavenging capacity; the lower the IC<sub>50</sub> value, the higher the free radical scavenging capacity) by processing method. Horizontal lines represent the mean TCC, TMC, and IC<sub>50</sub> values.

infusions rated with the lowest color intensity had the highest free radical scavenging capacity.

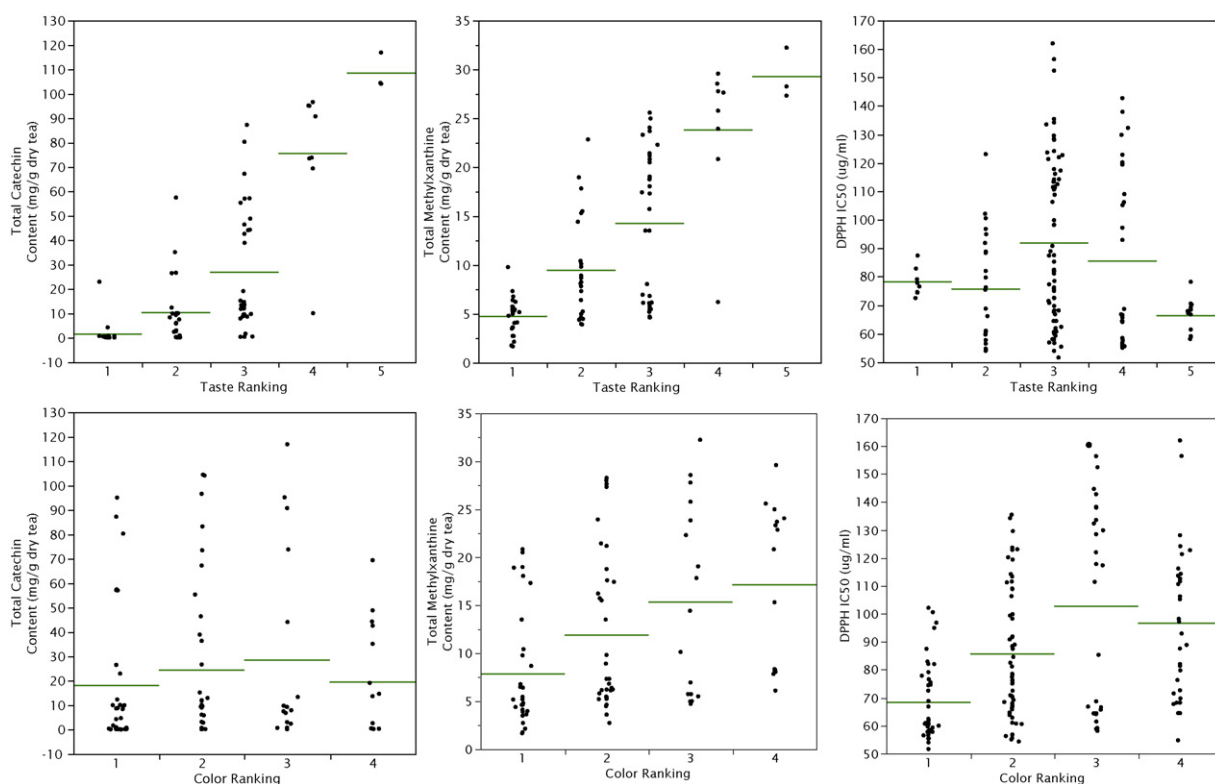
Significant variation was found for taste rankings and TCC ( $P < 0.0001$ ), TMC ( $P < 0.0001$ ), and IC<sub>50</sub> ( $P < 0.0059$ ). Means comparisons of TCC and TMC for all pairs of taste rankings found significant variations between most taste rankings ( $P < 0.01$ ). The exceptions were between: (1) levels 1 and 2 for TCC ( $P < 0.4558$ ), (2) levels 4 and 5 for TCC ( $P < 0.0743$ ), and (3) levels 5 and 4 for TMC ( $P < 0.6347$ ). Means comparisons of IC<sub>50</sub> for all pairs of taste rankings only found significant variation between level 3 and level 5 samples. Significant variation was found for color rankings and TMC ( $P < 0.0009$ ) and IC<sub>50</sub> ( $P < 0.0001$ ), while no significant variation was found for



**Fig. 5.** Mean quantities of individual analytes quantified by High Performance Liquid Chromatography (HPLC) per infusion by processing method.



**Fig. 6.** Box plots of TCC (Total Catechin Content), TMC (Total Methylxanthine Content), and IC<sub>50</sub> values (free radical scavenging capacity) by infusion sequence.



**Fig. 7.** Mean of TCC (Total Catechin Content), TMC (Total Methylxanthine Content) and  $IC_{50}$  values (free radical scavenging capacity) by taste and color intensity ranking. Horizontal lines represent mean values.

color and TCC ( $P < 0.6804$ ). Means comparisons of TMC for all pairs of color rankings found significant variations between levels 1 and 3 ( $P < 0.0018$ ), and levels 1 and 4 ( $P < 0.0122$ ). Mean comparisons of  $IC_{50}$  for all pairs of color rankings were significant except between levels 2 and 4 ( $P < 0.2107$ ), and levels 3 and 4 ( $P < 0.7952$ ).

#### 4. Discussion

##### 4.1. Perceptions of pu-erh tea tasting

The dichotomies of nature and culture, bitter and mellow, and raw and cooked (Levi-Strauss, 1997) resonate with informants' perceptions of pu-erh and can be linked with cultural identity and environmental history. Key informants perceived green or 'raw' (*sheng*) pu-erh samples to be bitter. 'Cooked' (*shu*) pu-erh samples were perceived as smooth tasting. The bitterest tasting green pu-erh samples were discerned as sourced from 'ecological,' 'natural,' or forest production environments that lack the input of pesticides, herbicides, and fertilizer. Rural upland communities in southern Yunnan have customarily produced and consumed bitter loose green pu-erh. Alternatively, 'cooked' or black pu-erh was consumed in urban areas. Urban tea drinkers developed a palate for artificially-aged pressed black pu-erh tea in the 1970s during China's drive towards modernization, away from the 'backwardness' associated with rural mountain cultures. However, the global urban green-food movement and attention to health-centered diets have revitalized a taste for bitter, 'ecological' and 'natural' pu-erh, now a prized commodity among urban tea drinkers.

##### 4.2. Variation of phytochemical constituents and free radical scavenging capacity

This study contributes to the literature on the variability of production and preparation practices of tea on TCC, TMC, and anti-oxidant activity profiles (Lin et al., 2003; Peterson et al., 2004;

Unachukwu et al., 2010). The values of TCC and TMC of aggregate infusions of individual samples are in accordance to previous studies on pu-erh that use protocols involving a longer extraction time (Wang et al., 2010). However, the 10th infusion of each sample indicates that the TCC and TMC have not been exhausted and that additional compounds remain to be extracted during additional infusions. These findings help validate the effectiveness of *gong fu cha dao* preparation in gradually extracting tea compounds. Future studies should employ a preparation protocol of a greater number of infusions.

The higher TCC and free radical scavenging capacity of green pu-erh samples compared to aged green and black pu-erh samples are consistent with previous studies on a decrease in polyphenolic levels and anti-oxidant activity of pu-erh and other teas with an increase in processing and aging (Qian et al., 2008; Ku et al., 2010; Wang et al., 2010). The variability between samples based on production and processing highlights the importance of sampling protocols to include a range of production variables. This study was limited in quantifying 12 analytes and did not account for the oxidative products, polysaccharides, and pigments formed during the processing from green to black teas (Wang et al., 2010). These compounds also have free radical scavenging capacity and may contribute to the greater variation in free radical scavenging capacity of black pu-erh tea samples compared to their TCC.

##### 4.3. Relationship of phytochemical profiles and perceptions of pu-erh tea tasting

The significant variation for TCC, TMC, and  $IC_{50}$  by taste rankings supports the role of taste in recognizing phytochemical properties of tea. Informants' perceptions that bitter taste profiles of pu-erh indicate medicinal attributes are in accordance with previous studies on the taste of constituents in tea (Rouseff, 1990; Ding et al., 1992; Drewnowski and Gomez-Carneros, 2000). Key experts' per-



ceptions of medicinal properties of pu-erh coincided with samples that contained the greatest TCC, while perceptions of stimulant properties of pu-erh did not coincide with TMC results. However, key experts' categorization of samples based on taste did show significant mean differences in TMC. Key experts' descriptions of green pu-erh samples as bitter and pressed black pu-erh as smooth tasting, coupled with insignificant mean TMC between samples, suggests that the bitter taste of pu-erh tea is more related to catechin than methylxanthine compounds. Casagrande (2000) suggests that bitterness alone may be inadequate for predicting pharmacological properties because human taste reception is not adequate to cognitively link the vast diversity of bitter chemicals with appropriate biological properties.

## 5. Conclusion

This study demonstrated that production environment, processing methods, and infusion sequence in preparing tea are related to the phytochemical profile, anti-oxidant activity, and flavor of tea. Findings contribute to the ethnomedical literature by supporting previous studies that have hypothesized that the taste of plants, particularly bitterness, may guide societies in the search for medicinal plants and beneficial phytochemicals. Results showed that the sensory experience of pu-erh tea, as quantified by ranking of taste and color, were correlated to phytochemical profiles and free radical scavenging capacity. Key experts' perceptions of the potential health benefits of tea were related to bitter taste and samples that contained the highest TCC. In addition, findings validated that the *gong fu cha dao* style of brewing tea through multiple infusions gradually extracts compounds as informants reported.

Nelson et al. (2002) suggest that the ability to recognize and respond to a wide range of chemical constituents is biologically relevant with significant evolutionary implications. As applied to pu-erh, consumer demand for 'bitter tea,' 'ecological tea,' and 'big tree tea' during pu-erh tea tasting may serve to link human with environmental wellbeing. The samples that had the greatest TCC were perceived by key experts as most bitter and were associated with the potential health benefits of tea. Key experts recognized these bitter tasting samples as being sourced from agro-forests rather than terrace plantations. Numerous studies have documented environmental implications of monoculture plantations and the benefits of low intensive agriculture, such as agro-forests. For example, Wang et al. (2010) found lead content in tea samples from terrace tea plantations in China exceeded the government's allowable standard by 35 times. Alternatively, traditional low intensive agrarian practices are considered to contribute to ecosystem services, plant species conservation, economic risk reduction, and food security (Harlan, 1975; Padoch and Peters, 1993; Moguel and Toledo, 1999; Belcher et al., 2005; Potvin et al., 2005; Jarvis et al., 2007; Jose, 2009). While the potential health-promoting constituents of tea have been widely studied, little attention has been given to the implications of tea from variable agro-ecosystems on human health. Future studies are needed to comprehensively examine how agricultural conditions affect tea flavonoid and methylxanthine profiles and associated bioactivity.

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