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Processing and chemical constituents of Pu-erh tea: A review

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ABSTRACT

Pu-erh tea is a unique microbial fermented tea produced from the sun-dried leaves of large-leaf tea species (*Camellia sinensis* (Linn.) var. *assamica* (Masters) Kitamura) in the Yunnan province of China. Pu-erh tea has become increasingly popular in Southeast Asia may be due to its multiple health benefits. The special sensory characteristics of Pu-erh tea arise from the multitudinous chemical changes and transformations of the chemical constituents of the sun-dried green tea leaves that occur during the post-fermentation process. Many functional components have been isolated from Pu-erh tea and identified. In this paper, modern processing techniques and their effects on the transformation of the chemical constituents and the major chemical components of Pu-erh tea are reviewed and discussed.

1. Introduction

Tea is one of the most popular beverages consumed in the world and its biological activities and health benefits have been widely explored (Lin & Lin-Shiau, 2006). It is normally produced from the leaves of two varieties of the plant *Camellia sinensis*: var. *sinensis* and var. *assamica* (Chen, Yu, & Tong, 2000; Wang, Provan, & Helliwell, 2000). Based on the processing procedures utilised, tea can be divided into green tea (non-fermented), oolong tea (semi-fermented), black tea (fully fermented by oxidizing enzyme) and dark tea (post-fermented by microbe). Unlike green tea, oolong tea or black tea, dark tea is a unique microbial fermented tea, and its production is only limited to the areas of China and Japan (Jiang et al., 2011). In China, dark tea is traditionally further divided into Yunnan Pu-erh tea, Hunan Fu-zhuan tea, Hubei Qing-zhuan tea, Sichuan Bian-xiao tea and Guangxi Liu-bao tea according to the different producing areas and the processing technologies employed. Among these teas, Pu-erh tea is of special concern, and is also the most representative dark tea. This tea has attracted more and more attention worldwide as a "hot topic" in the recent field of tea science.

Currently, Pu-erh tea is becoming increasingly popular especially in Southeast Asia mainly due to its multiple health-promoting effects (Mok, Chang, Wang, & So, 2008) as well as its special flavour and taste (Ahmed et al., 2010; Liang, Zhang, & Lu, 2005; Lv, Lin, Tan, & Guo, 2012; Lv, Zhong, et al., 2012). Pu-erh tea is favoured by an increasing number of consumers and therefore its market demand has expanded greatly. Because the consumption of Pu-erh tea has rapidly increased, the last decade has witnessed the rapid development of the Pu-erh tea industry and its escalated production. The Pu-erh tea output peaked in 2007 at approximately 99 thousand tonnes, which was almost a 9-fold increase compared to that of 2001, and accounted for 58.2% of the total Chinese dark tea produced (Tang, 2008). Microbial fermentation plays a crucial role in the formation of the special characteristics of Pu-erh tea, which result from dramatic changes in the chemical components of the tea leaves. However, its modern processing technology and the types of Pu-erh tea are little known outside China. In addition, reports of the chemical components of Pu-erh tea as well as other types of dark tea are limited (Ling et al., 2010).

Studies of the processing technology as well as the chemical constituents of Pu-erh tea have progressed in the last several years. With the rapidly growing popularity of this unique tea, it is important to review the recent advances comprehensively. Therefore, the present paper attempts to assess the developments in Pu-erh tea production and describe the chemical constituents of Pu-erh tea.

2. The definition of Pu-erh tea and its types

2.1. The definition of Pu-erh tea

Pu-erh tea is a unique microbial fermented tea. This tea is produced using the sun-dried leaves of large-leaf tea species (*C. sinensis* (Linn.) var. *assamica* (Masters) Kitamura) in the Yunnan province of China. The solid-state fermentation by microorganisms provides the special

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characteristics of a mellow taste, a stable flavour and the brownish red colour of the Pu-erh tea infusion.

As a well-known tea from ancient times with an interesting history, Pu-erh tea initially formed during long-distance transport of sun-dried green tea on horseback (Zhou, Zhang, Xu, & Yang, 2005), and the very path came to be known later as the famous Chinese Tea-horse Road (Shao, Powell, & Clifford, 1995a). Presently, Pu-erh tea is a national product of geographical indication and the producing areas are confined to certain regions in Yunnan province, including 11 cities, 75 counties, and 639 townships (specifically, between parallels 21°10′ and 26°22′ north latitude, 97°31′ and 105°38′ east longitude). The processing technology of solid state fermentation by microorganisms is widely used in the modern manufacture of Pu-erh tea, which has improved its manufacturing efficiency effectively.

2.2. Types of Pu-erh tea products and their relationships

Broadly speaking, the Pu-erh tea products on the market can be categorised into several types according to different standards (Yang, Chen, & Zhang, 2006), as shown in Fig. 1. First, Pu-erh tea can be divided into Pu-erh raw tea and Pu-erh ripen tea according to the processing technology and the quality characteristics (Fig. 1A). The former, a kind of green tea, is made directly from the sun-dried green tea by further autoclaving and compressing process, and its chemical constituents and quality are therefore very similar to those of the sun-dried green tea. Pu-erh ripen tea is normally made from the sun-dried green tea by microbial post-fermentation at higher temperature (about 50 °C) and higher humidity conditions. In addition, the compressed Pu-erh raw tea can be turned into Pu-erh ripen tea after natural ageing during long-term storage period, which is generally known as Pu-erh ageing tea. Secondly, Pu-erh tea can be divided into loose tea and compressed tea according to shape. After the autoclaving and drying processes, the compressed tea can be made by compressing the loose tea into different moulds to make different shapes (Fig. 1). Among the types of Pu-erh compressed tea, cake tea is the most common. Other desired shapes of compressed tea can be made as required, e.g., melon-shaped, tuocha-shaped, mushroom-shaped, brick-shaped and column-shaped (Fig. 1B).

Due to the domination of the production and consumption of the Pu-erh tea industry, Pu-erh tea in this paper generally refers to the microbial fermented Pu-erh ripen tea.

3. The manufacture process of Pu-erh tea

The modern manufacture process of Pu-erh tea is illustrated in Fig. 2. The material of sun-dried green tea is produced from the leaves of large-leaf tea species, *C. sinensis* (Linn.) var. *assamica* (Masters) Kitamura. Fresh tea leaves with one bud and two or three leaves are commonly used. After plucking, the fresh tea leaves are spread on bamboo mats for about 8 h in order to partially dry them. The tea leaves are then subjected to inactivation process of enzymes, by which they are fixed by heating in a drum to inactivate the endogenous polyphenol oxidase (PPO) (Liang et al., 2005). The temperature during the fixation process of the fresh green tea is much lower than that for making steamed, roasted and baked green teas. The lower temperature is considered to be in favour of the subsequent post-fermentation process. Because the deactivation of enzymes is incomplete (Ahmed et al., 2010) and partial enzyme activity is therefore retained. As a result, traces of theaflavins (TFs) could be detected in the obtained sun-dried green tea (Shao et al., 1995a) for the oxidative polymerization of catechins catalysed by PPO. The rolling time in the rolling process for Pu-erh tea is shorter than that for green tea, resulting in a relatively lower rate of the cell breakage rate and producing the loose rolled leaves, which facilitates their ventilation and the post-fermentation. Before the post-fermentation step, the rolled tea leaves are partially dried to give moisture contents of about 8% under the sun for 3 to 5 h at temperature above 30 °C. In the process of post-fermentation, the sun-dried tea leaves are piled up for a few weeks (Shao et al., 1995a), leading to a series of oxidation, condensation and degradation of tea chemical constituents, which are catalysed by the extracellular enzymes produced by microorganisms and thus to the production of the special sensory characteristics of Pu-erh tea. During the post-fermentation process, the leaves are turned over every two other days to prevent the excessive increase in temperature. Excessive post-fermentation induces the reduction of the antioxidant activity of Pu-erh tea (Zhang, Wang, Chen, Tan, & Wang, 2012) and thus should be avoided. After drying at below 60 °C following the post-fermentation (Hou et al., 2009; Wang, Gong, & Qiu, 2010; Wang, Xiao, et al., 2010), the loose Pu-erh ripen tea has been made and can then be compressed into different shapes after autoclaving and drying.

Many microorganisms are involved in the post fermentation of Pu-erh tea (Zhu, Yang, Li, & Zhang, 2008), including *Aspergillus niger*, *Penicllium*, *Rhizopus*, *Aspergillus glaucus*, *Saccharomyces*, *Aspergillus terreus*, *Aspergillus candidus* and *Bacterium*. Among these microorganisms, several *Aspergillus* spp. are regarded as the dominant species (Abe et al., 2008; Chen, Zhu, Wang, Zhang, & Yang, 2006; Zhao, Tong, Zhou, Wang, & Liu, 2010; Zhou et al., 2004). These microorganisms play a crucial role in the quality formation of the Pu-erh tea (Abe et al., 2008; Xu, Yan, & Zhu, 2005). The natural fermentation process normally takes a few weeks. In order to shorten the fermentation time, the dominant species of microorganisms are now extraneously inoculated in the modern processing technology (Chen, Chan, Chang, Liu, & Chen, 2009; Chen, Liu, & Chang, 2010; Hou, Jeng, & Chen, 2010; Liang et al., 2009). Research has revealed that artificial fungus-inoculated solid-state formation could give the main, specific and excellent sensory characteristics of Pu-erh tea, including the infusion colour, taste and aroma, which are comparable with spontaneous fermentation that produces the Pu-erh tea aged naturally for a long time (Chen, Zhang, Zhu, Yang, & Zhang, 2008; Liang et al., 2009). However, different microorganisms have different effects on the chemical compositions of Pu-erh tea (Fu, Song, Xu, & Li, 2012), such as the concentrations of lovastatin (Hao, Tong, Zhou, & Liu, 2012). Therefore, the selection of the microorganism stains used for the post-fermentation of Pu-erh tea is an important issue for the production of certain bioactive compounds and standardising the modern Pu-erh tea production. In addition, the discoveries of bioactive compounds and productions that account for the special characteristics of Pu-erh tea, which are responsible for health improvement and the aroma, colour and taste of the tea, respectively, will guild the production of high-quality Pu-erh tea.

4. Chemical components of Pu-erh raw tea

Pu-erh raw tea is produced from the leaves of *C. sinensis* var. *assamica* as sun-dried green tea form and its autoclaving compressed one. The chemical constituents of these two forms are very similar to one another and to those of fresh tea leaves, which have been identified by high performance liquid chromatography (HPLC) (Wang, Cheng, Zhou, Ye, & He, 2009), near infrared spectroscopy (NIRS) (Zhou et al., 2009), Fourier transform infrared spectroscopy (FTIR) (Ning, Zhang, Wang, Wan, & Zeng, 2011) and metabolomic analysis (Lee et al., 2011). However, the levels of these constituents are quite different because heat during autoclaving may induce the degradation of some tea chemical compounds.

To date, many compounds have been isolated and identified from the fresh leaves of *C. sinensis* var. *assamica* and Pu-erh raw tea (Hashimoto, Nonaka, & Nishioka, 1989; Zhang, Liu, Li, & Yang, 1995; Zhao, Ma, et al., 2011; Zhou & Yang, 2000). Among them, the flavonoids and the hydrolysable tannis are considered to be the major constituents possessing strong antioxidative properties (Gao, Zhang, Yang, Chen, & Jiang, 2008). As shown in Fig. 3, these

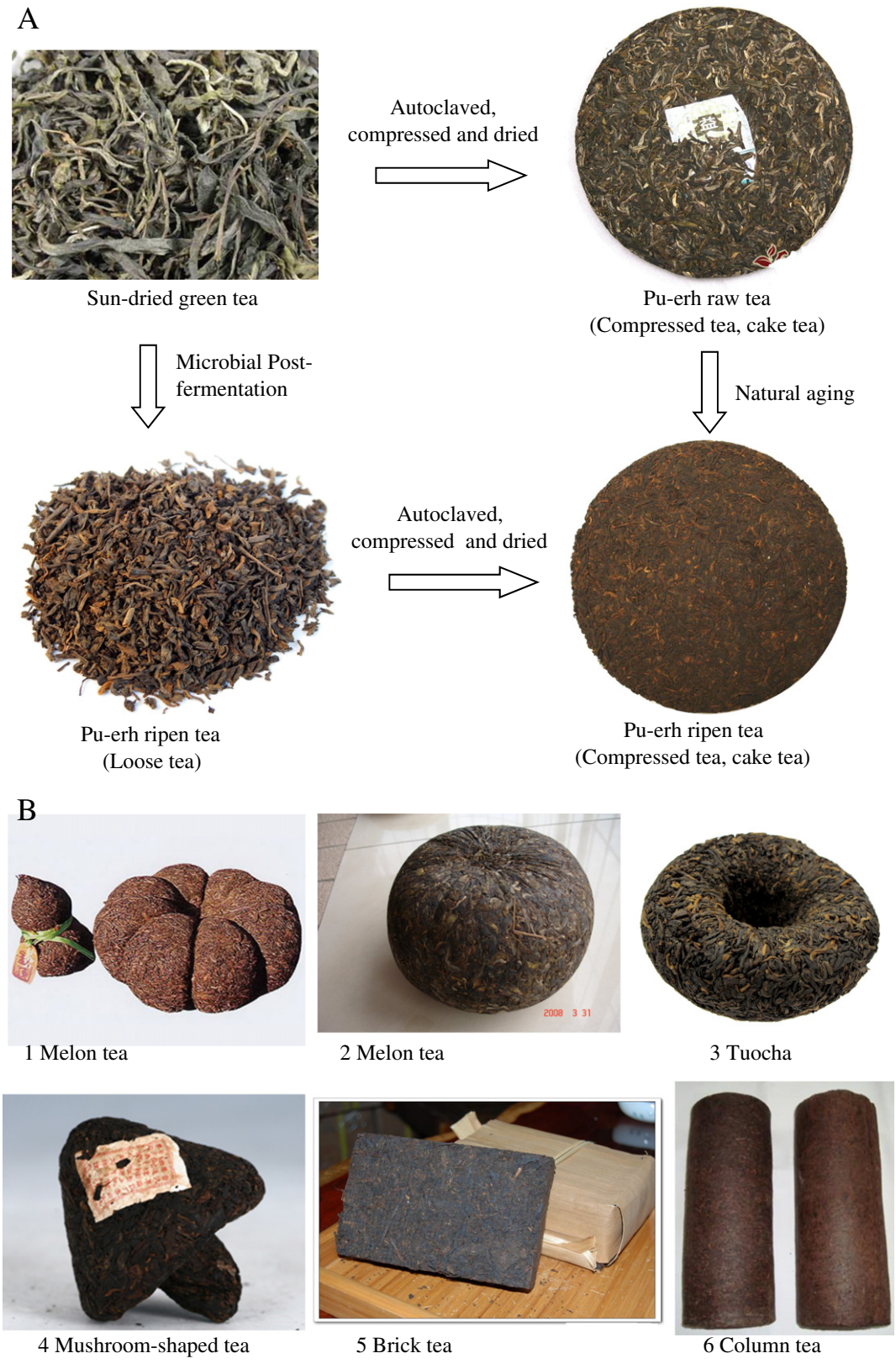

Fig 1. Different types and shapes of Pu-erh tea. (A. different types and their processing relations; B. various shapes of Pu-erh tea).

compounds can be divided into four categories according to their different chemical structures as follows:

(1) Flavan-3-ols and their derivatives. Similar to the case for regular green tea, the main chemical components of Pu-erh raw tea are catechins (epicatechin-3-*O*-gallate (ECG, **1**), epigallocatechin-3-*O*-gallate (EGCG, **2**), catechin (C, **3**), epicatechin (EC, **4**), epigallocatechin (EGC, **5**), catechin-3-*O*-gallate (CG, **6**), gallocatechin-3-*O*-gallate (GCG, **7**) and gallocatechin (GC, **8**)) as well as its derivatives (e.g., EGCG3″ Me (**9**), epiafzelechin-

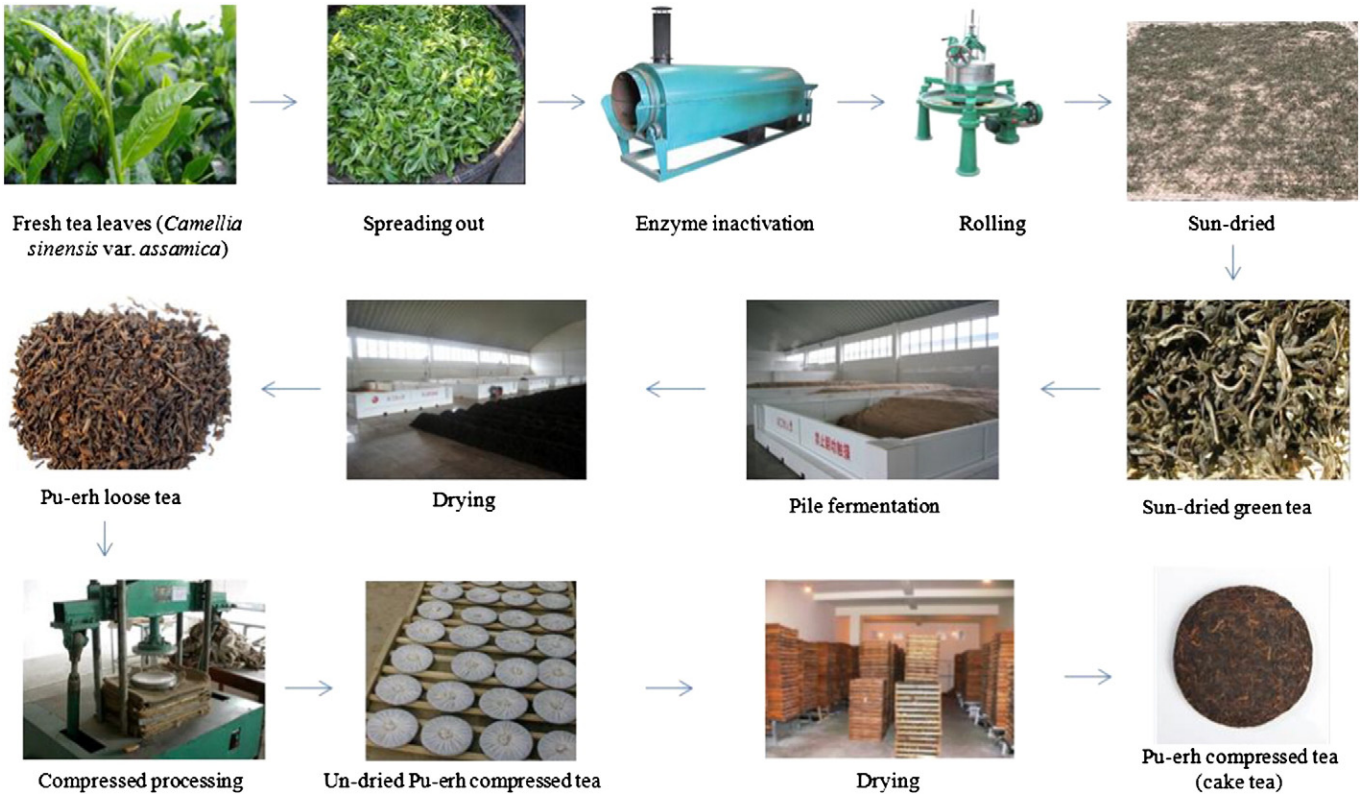

Fig. 2. Flow diagram of the modern manufacture process of Pu-erh tea.

3-*O*-gallate (**10**), epicatechin-3-*O*-benzoate (**11**) and epigallocatechin-3-*O*-caffeoate (**12**)), proanthocyanidins (e.g., gallocatechin-(4α → 8)-catechin-3-*O*-gallate (**13**), catechin-(4α → 8)-epigallocatechin (**14**) and gallocatechin-(4α → 8)-epicatechin (**15**)) and theasinensins (e.g., theasinensin A (**16**), theasinensin B (**17**) and desgalloyl theasinensin F (**18**)). Moreover, the fresh leaves of *C. sinensis* var. *assamica* were reported to contain chalcan–flavan dimers (e.g., assamicanins A (**19**)) and flavan 3-ol derivatives (Hashimoto, Nonaka, & Nishioka, 1989). Notably, the major catechins in sun-dried green tea are ECG (**1**), EGCG (**2**) and C (**3**), the most abundant of which is ECG (**1**) (Ahmed et al., 2010; Hou et al., 2009; Qian, Guan, Yang, & Li, 2008; Shao et al., 1995a; Wang, Gong, et al., 2010; Wang, Xiao, et al., 2010; Zhang, Li, Ma, & Tu, 2011). In contrast, EGCG (**2**) is generally the dominant catechin in the tea leaves of *C. sinensis* (Wu, Xu, Héritier, & Andlauer, 2012; Yang et al., 2007).

(2) Flavones and their derivatives. These compounds include mainly kaempferol (**20**), quercetin (**21**), and myricetin (**22**) and their *O*-glycosylated flavones as well as acylated and glycosylated flavones, however, apigenin was not detected in Pu-erh raw tea (Zhao, Ma, et al., 2011). The total flavone content of Pu-erh raw tea was 0.28 mg/g, whereas the total *O*-glycosylated flavone content was 11.75 mg/g. Thus, similar to other teas (Wang et al., 2000; Wu et al., 2012), the flavones in Pu-erh raw tea are also predominately present as glycosides rather than as non-glycosides. The quercetin glycosides are the major glycoside group in Pu-erh raw tea, including quercetin-3-*O*-rhamnosylgalactoside (**23**), quercetin-3-*O*-glucoside (**24**) and rutin (**25**). In addition, Pu-erh raw tea is also enriched in kaempferol glycosides, such as kaempferol-3-*O*-glucoside (**26**) and kaempferol-3-*O*-rutinoside (**27**) (Zhao, Ma, et al., 2011). The average content in three samples of Pu-erh raw tea was determined to be 2.54, 1.90 and 0.95 mg/g for rutin (**25**), quercetin-3-*O*-glucoside (**24**) and kaempferol-3-*O*-glucoside (**26**), respectively (Qian et al., 2008).

(3) Other phenolic compounds. These compounds include mainly simple phenolic acids, including gallic acid (**28**), caffeic acid (**29**) and *p*-coumaric acid (**30**) and their quininic acid derivatives (**31**–**39**), as well as hydrolysable tannins (**40**–**44**). The content of hydrolysable tannins, such as galloylglucose (**40**) and 1,2,6-trigalloylglucose (**42**), in Pu-erh raw tea is much higher than that of regular green tea (Zhao, Ma, et al., 2011) or Pu-erh tea (Ku, Kim, Park, Liu, & Lee, 2010). Furthermore, theogallin (**31**) is enrich in sun-dried green tea (Gao et al., 2008; Hou et al., 2009; Shao et al., 1995a; Wang, Gong, et al., 2010; Wang, Xiao, et al., 2010), and is abundant in the black tea produced in India and in Ceylonese black tea (Hashimoto, Nonaka, & Nishioka, 1992). Therefore, it is likely that theogallin (**31**) is a characteristic constituent of the large-leaf tea species. In addition, other compounds, such as coniferin (**45**), have also been identified in Pu-erh raw tea (Zhou & Yang, 2000).

(4) Alkaloids and their derivatives. These compounds include caffeine (**46**), theobromine (**47**) and theophylline (**48**). Generally, the content of caffeine (**46**) of Pu-erh raw tea has the highest values of all of the quantified alkaloids (Ahmed et al., 2010). The theobromine (**47**) content and theophylline (**48**) content of Pu-erh raw tea are much higher than those of regular green tea, whereas the caffeine content is found to be much lower (Zhao, Ma, et al., 2011).

Pu-erh raw tea has an even richer set of chemical substances than regular green tea (Chen, Yang, & Yu, 2004; Liu et al., 2012), including the water-extractable substances and tea polyphenols, which provides a favourable material base for the transformation of chemical constituents during the post-fermentation process and the natural ageing process. It was reported that a fingerprint identification method for Pu-erh raw tea has been established based on the HPLC profiles, by which Pu-erh raw tea can be distinguished from regular green tea (Ning, Zhang, Gu, Wan, & Sun, 2010). Moreover, the optimised ultra performance liquid chromatography (UPLC) techniques combined

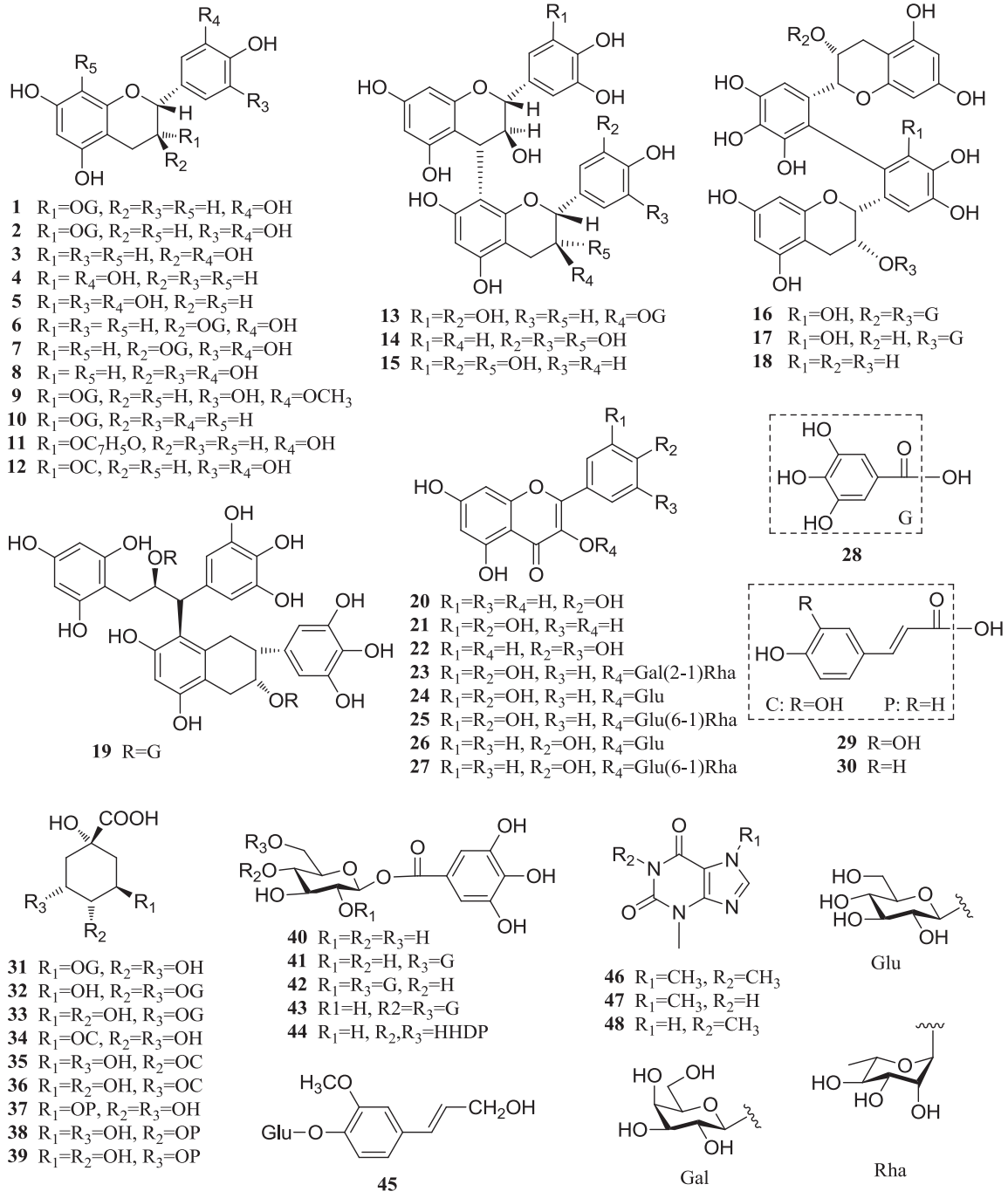

Fig. 3. Chemical components of Pu-erh raw tea.

with principal component analysis (PCA) were also reported to be capable of classifying and distinguishing between regular green tea and Pu-erh raw tea (Zhao, Ma, et al., 2011).

5. Chemical components of Pu-erh tea

The raw materials of Pu-erh tea come from sun-dried green tea rather than from fresh tea leaves. During the post-fermentation processing of Pu-erh tea, the chemical constituents in the sun-dried green tea have changed dramatically, and therefore some new chemical constituents that provide the unique quality of Pu-erh tea are formed (Gong, Zhou, Zhang, Song, & An, 2005; Luo, Wu, Deng, & Fu, 1998). The major compounds isolated and identified from Pu-erh tea are shown in Fig. 4.

(1) Flavan-3-ols and their derivatives. Pu-erh tea contains negligible amounts of catechins (Lin, Lin, Liang, Lin-Shiau, & Juan, 1998). The content of catechin monomers of Pu-erh tea is far less than that in green tea, oolong tea, white tea and black tea, and significant differences have been observed (Peterson et al., 2005). The residual catechins in Pu-erh tea are mainly GC (**8**) and EC (**4**) (Liang et al., 2005; Qian et al., 2008; Zhang, Li, et al., 2011), compared to EGCG (**2**) and GC (**8**) in green tea and GC (**8**) and ECG

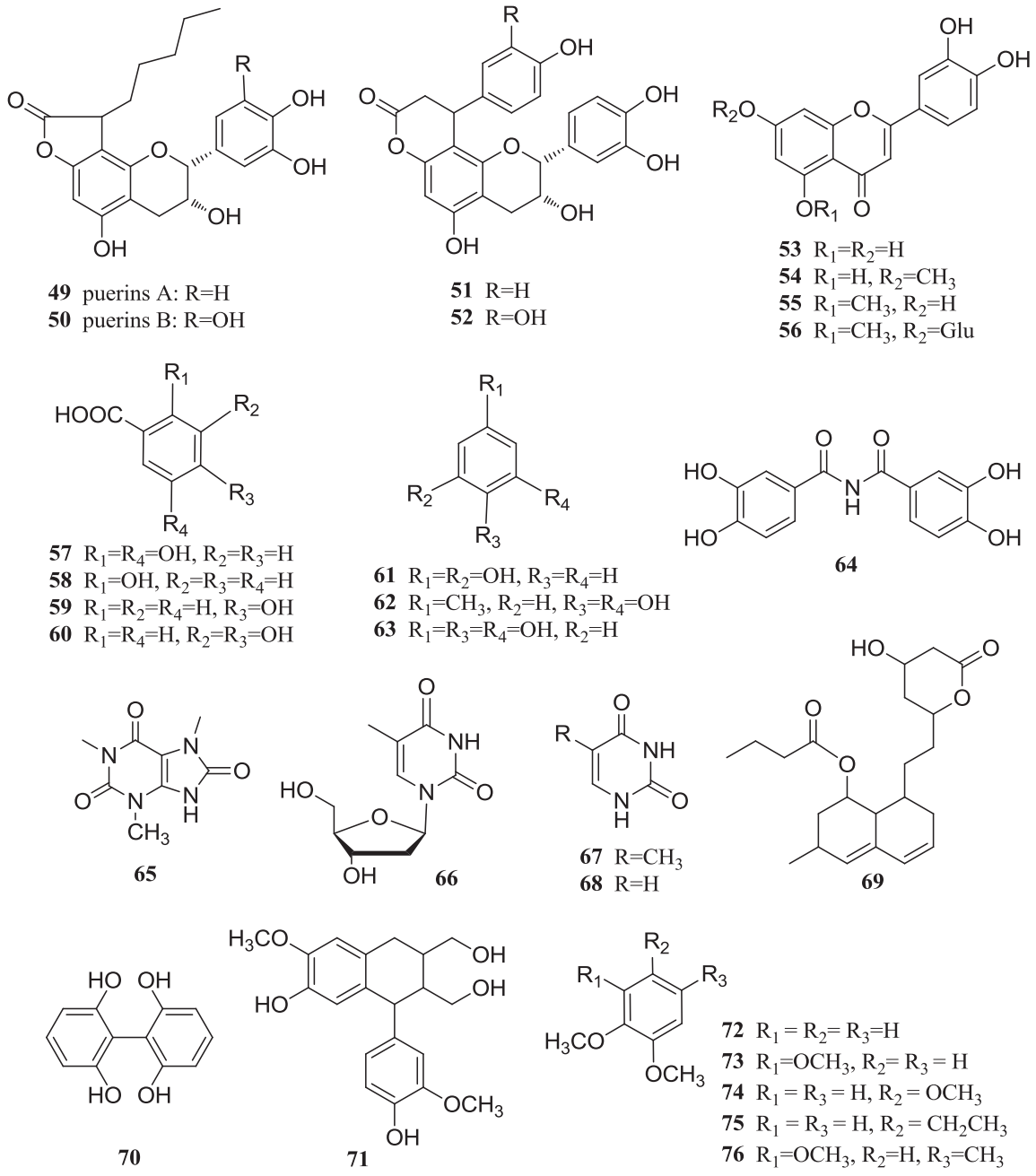

Fig. 4. Chemical components of Pu-erh tea.

(**1**) in black tea (Liang et al., 2005). The high content of GC (**8**) in Pu-erh tea may be the consequence of the biotransformation of tea catechins from EGCG (**2**) to EGC (**5**) and then to GC (**8**) (Tanaka et al., 2012). In addition, the main catechin in the Pu-erh tea extract has been reported to be EC (**4**) (Duh, Yen, Yen, Wang, & Chang, 2004). The contents of EGCG (**2**) and ECG (**1**) are relatively low and thus are hardly detectable by HPLC. Notably, two new 8-*C* substituted flavan-3-ols (puerins A (**49**) and B (**50**)) and two known cinchonain-type phenols (epicatechin-[7,8-bc]-4α-(4-hydroxyphenyl)-dihydro-2(3H)-pyranone (**51**) and cinchonain Ib (**52**)) have been identified in Pu-erh tea (Dong, Yang, He, & Lin, 2008; Zhou et al., 2005).

Theabrownin (TB), one of the main bioactive compounds in Pu-erh tea (Gong, Peng, Chen, Gao, & Zhou, 2010; Wang, Peng, & Gong, 2011), can be dissolved in water, but is not soluble in ethyl acetate, *n*-butyl alcohol or other organic solvents. TB is widely considered to be not only an essential factor in the unique colour and taste of Pu-erh tea, but also an important parameter for evaluating the quality of Pu-erh tea. The TB content in Pu-erh tea ranges from 10% to 14%, with an average content of 12% (Wang, Peng, et al., 2011). In addition, Xie et al. (2009) reported that the content of tea pigments on a dry weight basis in Pu-erh tea ranged from 0.16 to 0.29, 0 to 0.99, and 8.33 to 13.65% for theaflavins (TFs), thearubigins (TRs) and TB, respectively. Although on first examination Pu-erh tea appears superficially similar to black tea (Shao et al., 1995a) and the features of TB in both teas are also very similar, CP-Py-GC/MS analysis revealed major differences between the chemical constituents of Pu-erh tea and black tea (Gong, Tang, & Peng, 2012). Additionally, the Pu-erh tea brew was distinguishable from the black tea brew by the former lacking the TFs and

theaflavic acids that contribute to the astringency and bitterness of tea (Shao et al., 1995a; Shao, Powell, & Clifford, 1995b; Xie et al., 2009). However, although undetectable by HPLC, these compounds were detected by liquid chromatography–mass spectrometry (LC–MS) (Lin, Chen, & Harnly, 2008; Zhu, Clifford, Mao, & Deng, 2006).

(2) Flavones and their derivatives. The total flavonoid content in Pu-erh tea ranges from 0.88 to 0.96% (Zhang, Shan, & Zhou, 2008). The main flavones in the tea leaves are quercetin (**21**), kaempferol (**20**) and myricetin (**22**) (Lin et al., 2006). According to reports, the content of flavones on a dry weight basis in black tea leaves (Wang et al., 2000) ranges from 0.24 to 0.52, 1.04 to 3.03, and 1.72 to 2.31 g/kg, and in Pu-erh tea (Lu, Fan, Zhen, Ma, & Lan, 2006) ranges from 0.28 to 0.35, 1.45 to 1.72, and 1.10 to 1.12 g/kg for myricetin (**22**), quercetin (**21**), and kaempferol (**20**), respectively. These values compare well with the data reported by Li et al. (2012) except for the kaempferol (**20**) content of Pu-erh tea. However, in another report, the content of these three compounds was much lower and kaempferol (**20**) was barely detectable (Lu & Hwang, 2008). The discrepancies are most likely due to the different tea samples tested. The flavones in Pu-erh tea are predominately present as glycosides, and the major flavonoid glycosides in Pu-erh tea appear to be quercetin-3-*O*-glucoside (**24**) and quercetin-3-*O*-rutinoside (**25**) (Dong et al., 2008; Zhang, Li, et al., 2011). The flavonoid glycoside content in Pu-erh tea is much lower compared to that of Pu-erh raw tea. The average content of rutin (**25**) and quercetin-3-*O*-glucoside (**24**) in two Pu-erh tea samples was reported to be 0.43 and 0.46 mg/g, respectively (Qian et al., 2008).

Recently, four other flavones have also been identified in Pu-erh tea as follows: 3′,4′,5,7-tetrahydroxyflavone (luteolin) (**53**), 3′,4′,5-trihydroxy-7-methoxyflavone (**54**), 3′, 4′, 7-trihydroxy-5-methoxyflavone (**55**) and 3′, 4′, 7-trihydroxy-5-methoxyflavone-7-*O*-β-D-glucopyranoside (**56**) (Lv, Lin, et al., 2010). Among them, luteolin (**53**), which has been reported to have strong biological activity, was previously identified in Pu-erh tea in other studies (Peterson et al., 2005; Toyoda et al., 1997).

(3) Other phenolic compounds. Gallic acid (**28**) is a characteristic phenolic compound as well as one of the most important phenolic acids in Pu-erh tea with notable bioactivity (Sakagami & Satoh, 1997). The average content of gallic acid (**28**) in Pu-erh tea is 9.01 mg/g, but this varies widely between the different samples (Lv, Lin, Gu, Guo, & Tan, 2007). Other simple phenolic acids and their derivatives have also been identified in Pu-erh tea, such as 2,5-dihydroxy-benzoic acid (**57**) (Lin et al., 2006), 2-hydroxy-benzoic acid (**58**) (Zou et al., 2009), 4-hydroxy-benzoic acid (**59**), 3,4-dihydroxy-benzoic acid (**60**), 1,3-benzenediol (**61**), 4-methyl-1,2-benzenediol (**62**) and 1,2,4-benzenetriol (**63**) (Dong et al., 2008; Wang, Kadota, Liu, & Liu, 2005). It is worth noting that another microbial fermented tea, Fu-zhuan tea, is also rich in these phenolic acids and their derivatives (Fu et al., 2011). These compounds can further be chemically transformed into new compounds by the microbial activity. For example, 3,4-dihydroxy-benzoic acid (**60**) is a possible precursor of the new amide compound, *N*-(3,4-dihydroxybenzoyl)-3,4-dihydrobenzamide (**64**) (Zhang, Li, et al., 2011; Zhang, Ma, Che, Li, & Tu, 2011).

(4) Alkaloids and their derivatives. Similar to other teas, the major alkaloids in Pu-erh tea are caffeine (**46**), theobromine (**47**) and theophylline (**48**). Generally, caffeine (**46**) is the most abundant followed by theobromine (**47**) and theophylline (**48**) (Lin et al., 1998). Moreover, 8-oxocaffeine (**65**) and pyrimidine alkaloids (deoxythymidine (**66**), thymine (**67**) and uracil (**68**)) have also been isolated from Pu-erh tea, and they are now recognised as the new characteristic components of this tea (She, Chen, Zhang, & Yang, 2007). Because the microbial activity plays an essential role in the post-fermentation process, the formation of 8-oxocaffeine (**65**) likely arises from the biotransformation of caffeine. In contrast, deoxythymidine (**66**) might be derived from the combination of a pyrimidine alkaloid in the tea leaves and a microbial secondary metabolite.

(5) Others compounds and trace elements. Statins were first reported in Pu-erh tea by Hwang, Lin, Chen, Liuchang, and Shiao (2003). Lovastin (**69**) is the only statin in Pu-erh tea, and it exists in two forms in the water extract of Pu-erh tea (Yang & Hwang, 2006). Pu-erh tea is reported to have a lower content of amino acids than white tea and green tea. In Pu-erh tea, the theanine content ranges between 0.07 and 1.15 mg/g (Alcázar et al., 2007), and the natural fermented Pu-erh tea generally contains more theanine than the microbial fermented Pu-erh tea (Syu, Lin, Huang, & Lin, 2008). In addition, the γ-aminobutyric acid (GABA) content of Pu-erh tea is significantly lower than that of other types of tea, and thus, GABA is not a major bioactive constituent of Pu-erh tea (Zhao, Chen, et al., 2011). *N*-(3,4-dihydroxybenzoyl)-3,4-dihydrobenzamide (**64**), a new amide compound which was isolated and identified from Pu-erh tea, has been reported to be a very useful compound to prevent H_2O_2-induced cell death of Human micro-vascular endothelial cell (HMEC) (Zhang, Li, et al., 2011; Zhang, Ma, et al., 2011). In addition, 2,2′6,6′-tetrahydroxydiphenyl (**70**) (Zhou et al., 2005), isolariciresinol (**71**) (Wang et al., 2005) and the chlorophylls and carotenoids (Suzuki & Shioi, 2003) in Pu-erh tea have also been studied.

There are considerable amounts of methoxy-phenolic compounds (**72**–**76**) in Pu-erh tea (Cao & Liu, 2012; Lv, Lin, et al., 2012; Lv, Zhong, et al., 2012; Wang, Liu, et al., 2011; Wang, Peng et al., 2011; Zhou, She, Zhang, & Yang, 2006). These compounds are an important feature of Pu-erh tea as well as being among the key compounds contributing to the special aroma (Lv, Lin, et al., 2012; Lv, Zhong, et al., 2012; Xu et al., 2005), and are considered to be products of the methylation of gallic acid by microbial enzymes (Kawakami & Shibamoto, 1991; Kawakami, Kobayashi, Yamanishi, & Shoujaku, 1987; Yamauchi & Doi, 1997) and thermal degradation (Liu & Kazuo, 1987; Wang, Liu, et al., 2011; Wang, Peng, et al., 2011).

The ranges obtained for the elements analysed in 56 Chinese samples of Pu-erh tea were 80.2–151.6 mg kg^{-1} (fluoride), 0.66–4.66 mg kg^{-1} (lead), 14.8–19.3 mg kg^{-1} (copper), 1.95–4.98 mg kg^{-1} (chromium), 0.07–0.25 mg kg^{-1} (arsenic) and 0.023–0.130 mg kg^{-1} (cadmium) (Lv, Lin, et al., 2012). Moreover, the highest concentrations of ten of the fourteen studied elements (aluminium, barium, calcium, copper, iron, magnesium, manganese, nickel, phosphorus, potassium, sodium, strontium, sulphur and zinc) were found in samples of Pu-erh tea compared with black tea, green tea, Oolong tea and white tea (McKenzie, Jurado, & de Pablos, 2010). There was a significant difference in the fluoride content between the Pu-erh tea produced in Yunnan province (70.7 mg/kg) and the Bian-xiao tea produced in Hunan province (440.7 mg/kg) (Cao, Zhao, & Liu, 1998; Yi & Cao, 2008).

Although the beneficial health effects of Pu-erh tea are well documented, it is by knowing the chemical constituents that the health-promoting effects of the tea can best be justified (Mok et al., 2008). Unfortunately, because of the extraordinary complexity of its components, the bioactive constituents of Pu-erh tea are essentially unknown (Zhao, Chen, et al., 2011), which poses a major obstacle to the scientific elucidation of Pu-erh tea. Pu-erh tea is believed to contain special components that play the same role as EGCG (**2**) in terms of their beneficial effects on weight reduction (Wang, Gong, et al., 2010; Wang, Xiao, et al., 2010; Zhang, Li, et al., 2011; Zhang, Ma, et al., 2011). Encouragingly, some fractions that extracted and isolated from Pu-erh tea that contained no monomeric polyphenols, TFs and gallic

acid (**28**), exhibited antioxidant activity better than that of EGCG (**2**) (Jie et al., 2006). To find the key bioactive ingredients in Pu-erh tea and to develop these compounds for use in medicine and for health care purposes, systematic isolation, purification and identification of the chemical compounds of Pu-erh tea using modern phytochemical technology are warranted. Further studies of the bioactive constituents of Pu-erh tea are therefore crucial and urgently needed.

6. Conversion of the major chemical components during post-fermentation and natural ageing

6.1. Conversion of the major chemical components during post-fermentation

As shown in Table 1, at the end of pile-fermentation, the contents of tea polyphenols, catechins, TF, TR, amino acids, and soluble sugar were greatly reduced from the initial levels. On the contrary, the amounts of TB and insoluble polyphenols had clearly increased. At the same time, the total content of water-extractable substances was only slightly changed, suggesting that some new substances had formed from oxidation, degradation, and condensation of the various components, providing the special colour, taste and aroma to the Pu-erh tea (Luo et al., 1998). In addition, the polysaccharide level was substantially increased in Pu-erh tea (2.40%) compared with that of Pu-erh raw tea (0.35%), and the α-tocopherol level was also increased (9.12 mg/100 g in Pu-erh raw tea vs. 15.95 mg/100 g in Pu-erh tea), but fermentation did not affect the level of ascorbic acid (Hou et al., 2009). Research showed that tannase-treated green tea has a higher antioxidant capacity than its untreated analogue at a concentration of 200 ppm (Lu & Chen, 2008), suggesting that the tannase produced by the microorganisms during the post-fermentation process could change the bioactivity of Pu-erh tea.

Catechins, which are enriched in sun-dried green tea and impart bitterness and astringency to tea infusions, are dramatically reduced in Pu-erh tea (Lin et al., 1998). The large decrease in the catechin content results from the fact that the catechins have undergone a series of oxidative, condensing and degradative chemical processes to form TF, TR, and TB. In addition, catechin gallates are sensitive to thermal degradation (Zuo, Chen, & Deng, 2002). Gallic acid (**28**) is one of the most prominent phenolic acids in tea, and its content increased sharply during the post-fermentation process (She et al., 2005). It is likely that microbial activity promoted the formation of gallic acid (**28**) by hydrolysing the tannins in Pu-erh tea (Mukherjee & Banerjee, 2004; Van Diepeningen et al., 2004). Additionally, its derivation from the catechin gallates under the particularly high-temperature and high-moisture conditions was most likely another important reason for the high gallic acid content (Ananingsih, Sharma, & Zhou, 2011; Macedo et al., 2012; Tanaka et al., 2012; Zuo et al., 2002).

As shown in Table 1, the post-fermentation process increased the caffeine (**29**) content in Pu-erh tea (Tian et al., 2011). This result was confirmed by Wang, Wan, Hu, and Pan (2008), where they revealed that the change in the caffeine (**29**) content of tea leaves during the pile-fermentation depended not only on the growth and reproduction of microorganisms, but also on the tea composition. In addition, the amounts of myricetin (**22**), quercetin (**21**) and kaempferol (**20**) in the final Pu-erh tea products had decreased by approximately 50%, 62%, and 70%, respectively, compared with those of sun-dried green tea. The content of total flavone glycosides also decreased significantly during post-fermentation (Li et al., 2012). However, the flavonoid levels were substantially increased (Wang, Gong, et al., 2010; Wang, Xiao, et al., 2010; Zhang et al., 2008). It was reported that short-term fermentation of tea with *Streptomyces bacillaris* or *Streptomyces cinereus* enhanced the content of satins (**69**) (Jeng, Chen, Fang, Wei Hou, & Chen, 2007), substances that can hardly be detected in sun-dried green tea (Yang & Hwang, 2006). The changes in major chemical constituents described above contributed to the inevitable transformation of the taste quality from the heavy thick taste of sun-dried green tea to the stale mellow taste of Pu-erh tea.

Table 1
Change of the major chemical constitutes content, colour and antioxidant activity during the post-fermentation process.

Chemical constituents	Sun-dried green tea	Pu-erh ripen tea	Reference
Insoluble polyphenols	3.52%	6.38%	Luo et al., 1998
Tea polyphenols	29.73%	9.40%	
Total catechins	18.31%	3.62%	
EGC	2.15%	/	
C	0.49%	0.33%	
EC	1.19%	0.36%	
EGCG	4.04%	/	
ECG	11.31%	/	
GC		0.47%	Liang et al., 2005
GA	0.10%	1.77%	She et al., 2005
TF	0.14%	0.03%	Luo et al., 1998
TR	4.35%	0.20%	
TB	2.90%	7.85%	
Amino acid	1.80%	0.66%	
Theanine	0.40%	0.08%	She et al., 2005
GABA	13.0 mg/100 g	3.0 mg/100 g	Zhao, Chen, et al., 2011
Soluble sugar	5.13%	3.86%	Luo et al., 1998
Polysaccharides	0.35%	2.40%	Hou et al., 2009
Water-extractable substances	45.0%	41.0%	Luo et al., 1998
Caffeine	4.19%	5.17%	Tian et al., 2011
Flavonoids	0.89%	0.96%	Zhang et al., 2008
Kaempferol	1.26 mg/g	0.48 mg/g	Li et al., 2012
Quercetin	4.37 mg/g	1.31 mg/g	
Myricetin	0.63 mg/g	0.33 mg/g	
Flavonol glycosides	15.71 mg/g	5.32 mg/g	
Lovastin	/	139.26 ng/g	Yang & Hwang, 2006
DPPH scavenging activity	87.90%	80.94%	Zhang et al., 2012
Scavenging activity of superoxide anion free	135.1%	45.9%	
Aroma constituents (relative amount percent %)			
Alcohols	33.48%	21.38%	Lv et al., 2009
Aldehyde	3.39%	6.53%	
Ketones	6.96%	11.10%	
Esters and lactones	5.58%	9.49%	
Hydrocarbons	31.15%	13.38%	
Acids	1.14%	0.62%	
Methoxy-phenolic compounds	8.42%	27.10%	
Infusion colour index			
Luminosity	84.18	54.45	Wang et al., 2010
Chromaticity index *a*	−5.28	−38.37	
Chromaticity index *b*	16.12	31.43	

In terms of the tea aroma constituents in Pu-erh tea, during the post-fermentation process, the content of alcohols and hydrocarbons decreases sharply, whereas methoxy-phenolic compounds, aldehyde, and esters increase significantly (Lv, Zhong, Wang, & Lin, 2009), contributing to the change of aroma quality from the fresh brisk flavour of sun-dried green tea to the special stale flavour of Pu-erh tea. Moreover, the colour index of luminosity, chromaticity index *a* and chromaticity index *b* changes from 84.18, −5.28, and 16.12 to 54.45, −38.37, and 31.43, respectively (Wang, Gong, & Qiu, 2010), which represents the colour transformation from the yellowish green of sun-dried green tea to the brownish red of Pu-erh tea. A possible transformation path of chemical constituents during post-fermentation is shown in Fig. 5.

During the fermentation, extracellular enzymes produced by microorganisms, such as tannase, play a large role in catalysing chemical reactions and therefore promoting the transformation of the key chemical components. Methylation of hydroxyl groups is one of the

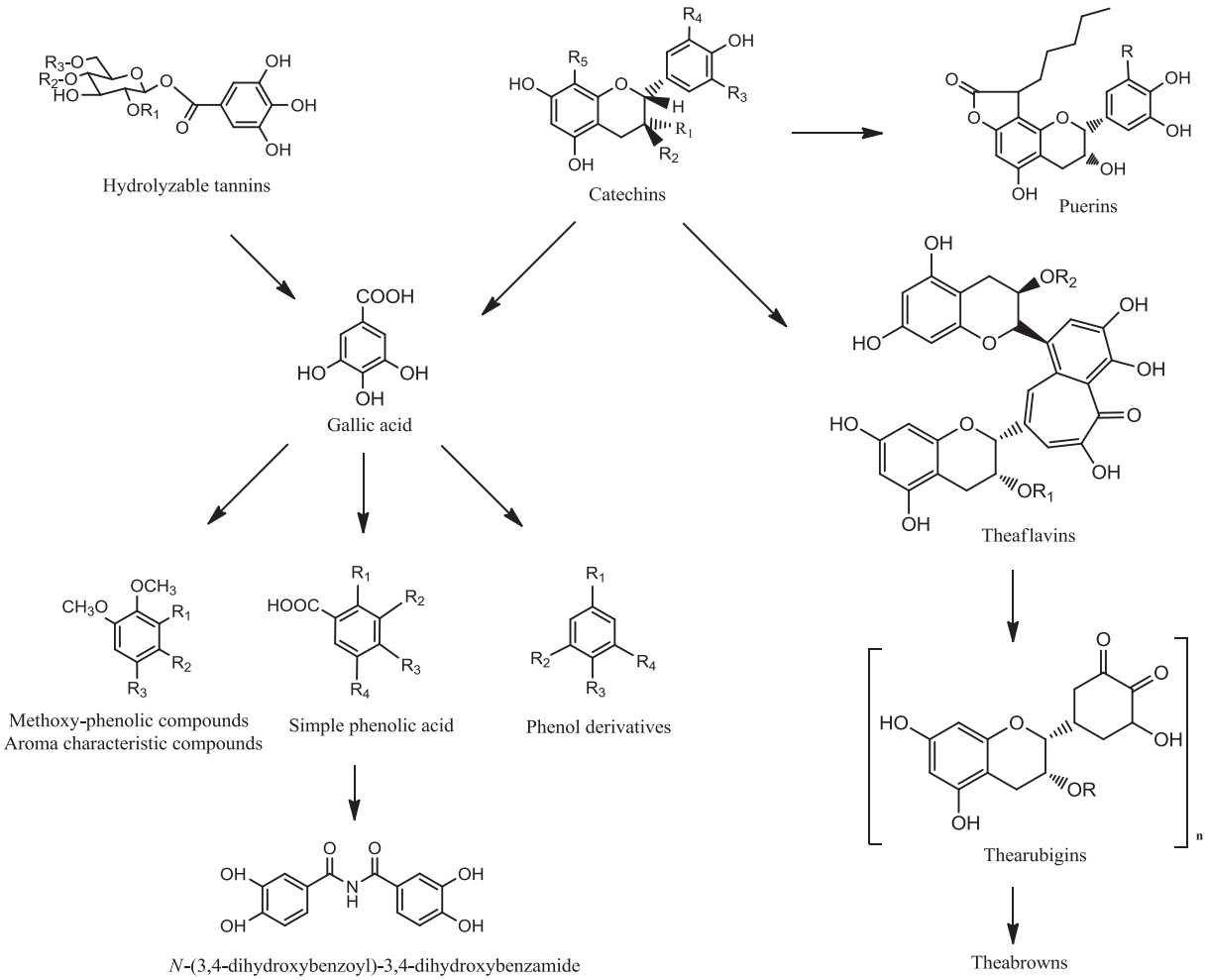

Fig. 5. Transformation of chemical constituents during the post-fermentation process.

major reactions requiring more study. A comprehensive investigation of the extracellular enzymes including tannase will clarify the key methylation enzyme and the role it plays. NMR-based metabonomics and proteomics are useful and powerful methods to employ in conducting this relevant research.

6.2. Conversion of the major chemical components during natural ageing

During the course of natural ageing, the chemical constituents in Pu-erh tea change and transform, albeit very slowly. It has been reported that the Pu-erh tea aged for 1 year and the Pu-erh raw tea aged for 6 years exhibited very similar waveforms and absorption peaks in their infrared spectrum, indicating that they share similar characteristic chemical components (Ning et al., 2011). However, the free radical scavenging activities decreased with the increase of the preservation period (Ku et al., 2010; Qian et al., 2008). Significant correlations between certain chemical compounds (such as EGCG (**2**), strictinin (**44**) and gallic acid (**28**)) and the length of the post-fermentation period have been observed in Pu-erh raw tea. The amounts of these compounds changed in a year-dependent manner, and hence, they could serve as biomarkers for determining the age of Pu-erh raw tea (Ku et al., 2010).

However, methods to predict the age of Pu-erh tea are still technically challenging. A metabolomic approach based on LC–MS (Ku et al., 2010) or ultra performance liquid chromatography–quadrupole time-of-flight mass spectrometry (UPLC–QTOFMS) (Xie et al., 2009) and combined with multivariate analysis was shown to have potential as a useful tool for determining the post-fermentation year. For example, a two-component PLS score plot of the UPLC–QTOFMS data could depict the general chemical variations in Pu-erh teas of different ages, and the PLS score plot could be readily separated by PC1 (Xie et al., 2009). The report has also revealed that 1-year-old and 3-year-old Pu-erh tea samples differed in their concentrations of certain chemical constituents and they both differed from the 5-, 8-, and 10-year-old Pu-erh tea samples. However, the separation of 5-, 8-, and 10-year-old Pu-erh tea was less clear, indicating that the quality of Pu-erh tea stabilised after a given period. Although progress has been made in analysing and judging the different types of Pu-erh tea by FTIR (Xu, Deng, & Cai, 2011), metabolomics (Ku et al., 2010) and HPLC-DAD–ESI–MSn (Zhang, Li, Ma, & Tu, 2011), these technologies are still not accurate enough to predict the age of the tea because of the complexity of the mixture of sun-dried green tea materials.

7. Future studies of Pu-erh tea

To make better use of Pu-erh tea and attract more attention to the scientifically proven benefits of drinking Pu-erh tea, more studies will be conducted in recent future. The main trends in this field might include the following. (1) To investigate the bioactive compounds responsible for the reported health benefits of Pu-erh tea and the mechanisms for the formation of these bioactive compounds. (2) To investigate reasonable consumption levels for different groups of Pu-erh tea drinking.

(3) To study the extracellular enzymes produced by microorganisms. (4) To explore the mechanisms that produce the distinctive qualities of Pu-erh tea. (5) To standardise and optimise the Pu-erh tea processing technology. (6) To predicate the age of Pu-erh tea using modern technology.

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