Chapter 15
Yunnan Pu-erh Tea

Jiashun Gong, Qiuping Wang, Sarote Sirisansaneeyakul, and Anna McElhatton

Contents

J. Gong (✉)
Faculty of Food Science and Technology, Yunnan Agricultural University,
Kunming 650201, P.R. China
e-mail: gong199@163.com

Q. Wang
Faculty of Food Science and Technology, Yunnan Agricultural University,
Kunming 650201, P.R. China

Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University,
50 Ngam Wong Wan Road, Chatuchak, Bangkok 10900, Thailand
e-mail: soffywang87@gmail.com

S. Sirisansaneeyakul
Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University,
50 Ngam Wong Wan Road, Chatuchak, Bangkok 10900, Thailand
e-mail: sarote.s@ku.ac.th

A. McElhatton
Faculty of Health Sciences, University of Malta, Msida, Malta
e-mail: anna.mcelhatton@um.edu.mt

A. McElhatton, M.M. El Idrissi (eds.), *Modernization of Traditional Food Processes and Products*, Integrating Food Science and Engineering Knowledge Into the Food Chain 11, DOI 10.1007/978-1-4899-7671-0_15

15.1 Introduction

15.1.1 History of Yunnan Pu-erh Tea in China

During Ming Dynasty (1368 C.E.–1644 C.E.), Kunming Taihua tea, Dali Gantong temple tea and Wanding (Changning country) tea were the most known teas of the period. Pu-erh tea has a much longer history that can be traced back to the Eastern Han Dynasty (25 C.E.–220 C.E.). In those times, tea was processed close to where it was cultivated and shipped directly to markets. There were various varieties such as "scissors crude tea" from Yongning (now known as Ninglang county) which got its name because it was actually harvested using scissors, "Pu-erh tea" from Cheli (presently known by the names of Pu-erh county and Xishuangbanna) and "Wumeng tea" from Wumeng (presently Zhaotong city).

"Pu-erh tea" was the dominant variety and was widely distributed in Yunnan province. "DianhaiYu Hengzhi", a book written in the 4th-year Jiajing (1525 C.E.) of Ming Dynasty, describes the prosperity that the tea trade brought and provides a description of the trade:

> The six Tea Mountains for producing Pu-erh tea are Youle, Gedeng, Yibang, Mangzhi, Manzhuan and Mansa. Hundreds of thousands people work on the Tea Mountains to harvest and process Pu-erh tea. Then the Pu-erh tea is bought and transported to the other places by the tea merchants on the horsebacks. All of the roads are full of caravans, and the tea merchants make a tidy profit from the Pu-erh tea business.

In the 13th year (1748 C.E.) of Qianlong emperor during Qing Dynasty, the Tibetan people consumed large quantities of Yunnan tea, and the Yunnan tea sales to Tibet were so significant that the tea so traded was given its own identity and called Chitsu Pingcha. The Chitsu Pingcha was a unit of tea that consisted of a case of seven circles or cakes, with a weight of 49 Liang (equivalent to approximately 1.83 kg). According to the historical records, the annual sales volume of Chitsu Pingcha was said to reach 3000 picul (approximately 150 t) at that time. By 1825, Ruan Fu, a writer of Qing Dynasty, wrote in his book on Puerh tea that "this tea was well known globally". At the end of the nineteenth century (1894), the Yunnan province tea sales reached 1500 t. At that time, a fine variety of *Camellia sinensis* Kuntze. var. *assamica* Kitamura was initially introduced to many other areas of western Yunnan for plantation. In 1937, new tea-growing regions were extensively developed in Yunnan province. As a result, the entire province produced Pu-erh tea with harvests reaching some 9800 t annually. In 1940, the Yunnan tea sold to Tibet reached its peak of 40,000 packages (about 1.5×10^6 kg).

The history of Yunnan Pu-erh tea, also has ties with the well-known "Tea Horse Road" (Cha Ma Gu Dao) and The "Silk Road" that are both networks of land trading routes. The "Silk Road" connected north western China to Europe from the times of the Han dynasty (25 C.E.–220 C.E.), while the "Tea Horse Road" connected China to various parts of Asia and Europe before seafaring became. It should be noted that the "Tea Horse Road" is sometimes referred to as the "Silk Road of the south".

The "Tea Horse Road" got its name as Chinese tea and horses were the main products traded along the route (together with medicine, salt, cloth, and skins, mostly carried by mules). Historians have traced the origins of the "Tea Horse Road" back to the Tang dynasty (618 C.E.–907 C.E.), when tea was transported out of Yunnan to Beijing, Tibet, and other Southeast Asian countries. The "Tea Horse Road" was further developed during the Song (960 C.E.–1279 C.E.) and Ming dynasty (1368 C.E.–1644 C.E.), and remained a key trading route for Pu-erh tea and other commodities until the Qing dynasty (1644 C.E.–1911 C.E.). There were five routes of the "Tea Horse Road", they were specifically depicted as follows:

Guan Ma route, which ran from Pu'er to Kunming, and further to other provinces within mainland China. It was used for transporting imperial tea to Beijing.

Guan Zang route, which ran from Pu'er to Xiaguan, Lijiang, Shangri-la, and into Tibet; and from Tibet to Nepal and other countries.

Jiang Lai route, which ran from Pu'er to Jiangcheng, into Lai Chau of Vietnam, and onto Tibet and Europe.

Dry Season route, which ran from Pu'er to Simao in Yunnan, and onto the Lancang River, Menglian, and Burma.

Meng La route, which ran from Pu'er to Mengla in Yunnan, and onto northern Laos and Southeast Asia.

At that time, sun-dried green tea leaves were packed and shaped into cake, brick, mushroom, square and bowl shapes to be convenient for transportation. Transportation times lasted weeks if not months, as an example the transportation on the Guan Zang from Pu'er to Tibet, took some 100 days during which time the colour of the tea became darker, and flavour no longer astringent. This occurred because the packed green tea leaves underwent oxidation and fermentation during the trade journey; both processes occurred because of the interaction of the tea with moisture and due to temperature fluctuations. This caused the tea's organoleptic properties to gradually change such that they became highly desirable and therefore also so valued.

In the 1930s due to improved transportation, many new tea roads were opened into Myanmar, Laos and India. Meanwhile packaging and the storage conditions in warehouses improved significantly.

Due to the fact that improved transportation processes significantly reduced the time to reach Tibet had reduced from 100 days to 40 days natural fermentation that required longer process times was incomplete. As a consequence of this processing issue various tea processing factories began to artificially ferment Pu-erh tea. This led to the development of the modern manufacturing technique of Pu-erh tea by solid state fermentation (SSF).

15.1.2 Definition of Yunnan Pu-erh Tea and Its Types

Historically various kinds of tea handled by the Pu-erh local government were called the Pu-erh tea. Pu-erh tea had been originally produced in the Diannan Lanchan River basin, using crude sun-dried green tea prepared from the fresh leaves of *Camellia sinensis* var. *assamica*, through a process of "enzyme(s) inactivation of rolling-sun drying" technology to form raw sun-dried green tea and further to shaped and compressed each kind of compressed tea by heating with steam.

In 1973, the Yunnan tea import–export company first developed a new technique for processing modern Pu-erh tea in the Kunming Tea Factory and Menghai Tea Factory. The Chinese Classic Book of Tea stated that Pu-erh tea was produced from sun-dried green tea that then underwent SSF, and then shaped into tea bricks.

In 2008, Pu-erh tea was declared "a product with geographical indications", and the production areas clearly defined and confined to certain regions in Yunnan province specifically, between parallels 21°10′ and 26°22′ north latitude, 97°31′ and 105°38′ east longitude these designated areas included the fields, towns, cities and counties.

Broadly speaking, the Pu-erh tea products on the market can be categorized into several types according to different standards, as shown in Fig. 15.1. Firstly, Pu-erh tea can be divided into Pu-erh raw tea and Pu-erh ripened tea according to the processing technology and the quality characteristics (Fig. 15.1a). The raw tea, a kind of green tea, is made directly from the sun-dried green tea by further autoclaving and a compression process. Its chemical constituents and quality are 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 ripened tea after natural ageing during long-term storage which is generally known as Pu-erh aged 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. 15.1). 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. 15.1b). Currently, the dominant type of Pu-erh tea produced and consumed of is the microbial fermented Pu-erh ripe tea.

Microbial solid state fermentation is known to change the composition of the tea. Tea polyphenols, catechins, thearubigins and theaflavins decreased, while amino acids and the carbohydrates content are somewhat reduced. There is a development of fragrance compounds, many of which have mellow fragrance such as nonanal, linalool, oxidized linalool, 1-ethyl-2-formacyl pyrrole and phenylacetaldehyde are obviously high. In addition, gallic acid, 1,2-dimethoxy-4-methyl benzene, 1,2-dimethoxy-4-ethyl benzene and 1,2,3-trimethoxy-4-ethyl benzene noticeably increase. These characteristic profiles demonstrate that, Pu-erh tea should not belong to the block or brick tea, but should have its own tea variety designation because of the uniqueness of its characteristics.

Fig. 15.1 Different types and shapes of Pu-erh tea. ((**a**) Different types and their processing relations; (**b**) various shapes of Pu-erh tea). *Source*: Lv et al. (2013)

15.1.3 Pu-erh Tea as a Functional Food

Zhao Xueming, a writer during the Qing Dynasty, was cited in the Ben Cao Gang Mu (a Chinese herb pharmacopoeia), as describing Pu-erh tea as “mild and fragrant… it has incomparable perfume, which is very good for drinkers, helping in digestion and eliminating expectoration” (Chen et al. 2010).

A review by Zhang et al. (2005) has reported that Pu-erh tea has the potential to produce various physiological changes that include reduction of blood lipids, cholesterol and control of body weight, lowering of blood pressure and the control of arthrosclerosis.

15.1.3.1 Avoiding Cancer and Improving Anticancer Conditions

In addition studied the potential for Pu-erh tea effects on cancer cells. The tea polyphenols together with their oxidation and degeneration of resulting complex products in Pu-erh tea are all important components with anticancer potential.

In addition, Pu-erh tea prepared from fresh leaves of *Camellia sinensis* var. *assamica* is rich contains, like beta-carrot elements, vitamin B_1, B_2, C, E and so on. All are important compounds responsible for anticancer.

It is also reported to settle the stomach and to have antiageing effects.

15.2 Processing Techniques of Yunnan Pu-erh Tea

15.2.1 Traditional Techniques for Pu-erh Tea Processing

Although Pu-erh tea has a long cultural history technical information about Pu-erh tea related literature is limited. It is known that based on the picking or harvesting time and fresh leaf quality and type, Pu-erh tea can be divided into four main types, “the wool point”, “the tender tea-leaves”, “the Xiaomang tea” and “the valley flower-scented green tea”. The processing of these teas are different, subsequently they also differ. The finished products are described as tight round tea, female tea, Jin Yuetian and lump tea. A comprehensive description of the predecessors of all forms of Pu-erh tea processing is as shown in Fig. 15.2.

The Pu-erh tea produced in the traditional way acquires its shape due to the different types of basket used to transport and export it to places such as Tibet, Hong Kong and Myanmar. This traditional processing method keeps is still in use. Sun-dried green tea is used as raw materials; hence it is classified as green tea. The tea’s characteristics are developed through steam rubs, in which the temperature affects tea quality.

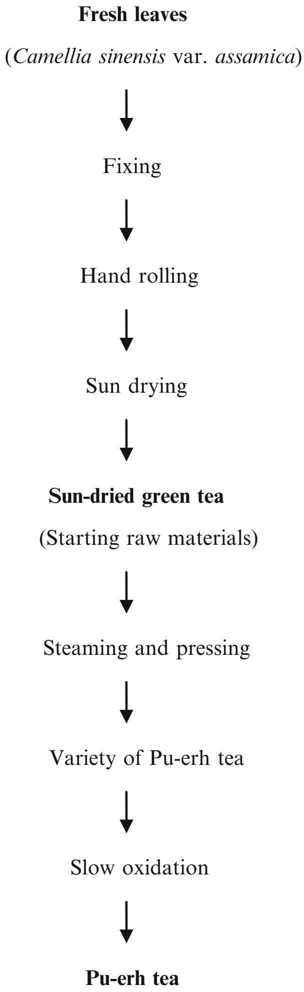

Fig. 15.2 The traditional procedure of processing Pu-erh tea

It is well accepted that the quality attributes were traditionally formed during the long transportation times. Therefore, Pu-erh tea quality is undoubtedly the product of the specific historical conditions.

15.2.2 Modern Techniques for Pu-erh Tea Processing

Economic change has motivated the development of the modern technique for the Pu-erh tea production. In the twentieth century, at the beginning of 1970s, the consumers' request for quality Pu-erh tea increased. The Yunnan Province tea companies began to ferment and reprocess Pu-erh tea such as had been done in the

Kunming tea factory and Menhai tea factory. Pu-erh tea processing entered the new development era. The whole process of studying Pu-erh tea, from raw materials, processing methods, chemical composition, quality testing, were developed and the scientific idea of modern Pu-erh tea was finally established.

The modern Pu-erh tea, using sun-dried green tea as raw materials, through splashing water, solid state fermentation, mellow stage and drying and so on, made Pu-erh tea a special kind of tea. The traditional characteristics of Pu-erh tea namely its mellow taste and Chen fragrant characteristic were retained in modern processing.

Pu-erh tea became widely available especially in Hong-Kong, Macao and Taiwan, and further afield in Southeast Asia and the volume of exports continue to increase. The microbial involvement in the process is complex and is the source of the characteristic tea quality that has been associated with its cultural history. Figure 15.3 describes the modern technical process of manufacturing Pu-erh tea. In this modern processing method, there are four stages that have evolved from the traditional craft link:

1. Pu-erh tea's raw materials, which are typically prepared from *Camellia sinensis* var. *assamica* of Yunnan Province. After enzymes inactivation of fresh tea leaves by hot steam, the steps of rolling tea leaves and drying them by sunlight are essential to obtain the sun-dried green tea.
2. Solid state fermentation (SSF), the SSF facilitates the slow oxidation of Pu-erh tea. The fermentation involving beneficial microorganisms which through their growth and metabolism induce characteristic biotransformation, forming the unique quality characteristics of Pu-erh tea.
3. Special climatic conditions, in Yunnan Province, naturally provide the optimal conditions for the requisite microbial secondary metabolism that facilitates the production of quality Yunnan Pu-erh tea. The sunshine in Yunnan appropriate for the production of Pu-erh tea, where the yearly average temperature ranges from 12 to 23 °C providing the solar energy to sun-dry green tea leaves.
4. Pressing and shaping, the hot and damp material is also thought to influence the quality of Pu-erh tea. The pressing and shaping may influence chemical transformation of ingredients to remove astringency and render the tea mellow in taste.
5. Storage and ageing, under controlled evidence based storage conditions, indicate that the longer the Pu-erh tea is aged the mellower in taste it becomes. The long storage time may cause changes in the chemical composition of Pu-erh tea through oxidation. Due to the fact that there is significant variability in the raw materials, the finished Pu-erh tea typically also varies and are given names such as the raw cake, "shengpu series" and the modern craft of the ripe cake, "shupu series", as well as "dry store Pu-erh tea" and "wet store Pu-erh tea" that are formed by different storage conditions. As a result, there are many kinds of Pu-erh tea.

Large fresh leaves in Yunnan Province

(*Camellia sinensis* var. *assamica*)

↓

Fixing

↓

Rolling

↓

Sun drying

↓

Sun-dried green tea

↓

Water spraying

↓

Inoculation with microorganisms

↓

Solid state Fermentation (SSF)

↓

(i) Unpacked Pu-erh tea, and/or (ii) Shaped Pu-erh tea (by pressing and shaping**)**

↓

Packaging

↓

Storage and aging

↓

Yunnan Pu-erh tea

Fig. 15.3 Modern procedure for producing Pu-erh tea

15.3 Research Progress in Chemical Composition of Yunnan Pu-erh Tea

The chemical composition of tea is the foundation of tea quality. So far, there are about 600 known compounds in tea of which more than 450 are organic compounds kinds. With the exception of carbohydrate, fat and lipid, found in the raw materials, the other compounds are all the secondary metabolites. Among tea's secondary metabolites, there are polyphenols and purines that account for 20–38 % and 3–5 % of the tea's composition, respectively. In general, tea colour, its smell taste, and the quantity and profile of the secondary metabolites are all factors affecting the decision regarding quality. The tea infusion quality is therefore an association of various attributes that include the taste of the tea infusion that is influenced by chemical components and the processing itself which gives the tea its full-bodied taste, its colouration (as shown in Fig. 15.4), and its Chen (aged) or mellow fragrance.

Fig. 15.4 Comparison between sun-dried green tea and Pu-erh tea after solid state fermentation, (**a**) sun-dried green tea, (**b**) Pu-erh tea after solid state fermentation, (**c**) Yunnan Chitsu Pingcha, as a kind of Pu-erh tea, (**d**) a water extract colour from sun-dried green tea and (**e**) a water extract colour from Pu-erh tea after solid state fermentation

15.3.1 Chemistry of Raw Materials

Pu-erh tea is prepared from sun-dried green tea as starting raw materials. After sun-drying the fresh leaves of *Camellia sinensis* var. *assamica*, a step of special solid state fermentation is required, then pressing and shaping is particular to produce a variety of Pu-erh tea. For sun-dried green tea, its production is mainly from the area of Yunnan national minority. The fresh leaves of *Camellia sinensis* var. *assamica* are collected by labour picking, inactivation of enzymes, rolled and cooled, and then dried with solar energy.

The moisture content of sun-dried tea leaves is approximately 10–12 %, which is much higher than that of fried green tea (approximately 6–8 %). The modern Pu-erh tea is basically evolved from the traditional Pu-erh tea. The typical characteristics are mellow taste and remarkable Chen fragrant. However, both are commonly so-called Pu-erh tea, neither traditional nor artificial Pu-erh tea is of necessity identified. With using big leaf planting tea as material and fermenting step result in the characteristic quality of brown colour and Chen fragrant. Since the natural fermentation is quite slow process for traditional Pu-erh tea, the fermentation time of modern Pu-erh tea is fast under artificially controlled conditions. Nevertheless, both the techniques form similarity of Pu-erh tea characteristic quality.

Pu-erh tea is well-known worldwide at the present time. The production and exporting volume of Pu-erh tea are increasing of great concern. The unique quality characteristics of Pu-erh tea are essentially formed, because particular chemical substances present in bud leaf and tender stem are typical, additionally the biochemical changes after the solid state fermentation process are of great importance.

Table 15.1 summarizes chemical components of various Chinese sun-dried green teas (Gong et al. 2005). They are especially rich in polyphenols and a number of different oxidized products depending upon the source of the leaves. The raw materials from platform (mesa) plantation area tea show higher contents of polyphenol, total catechins, oligosaccharides and total carbohydrates than those from old tea, except those of flavanones, where the theaflavins and thearubigins are actually lower. This has laid the foundation for the unique quality formation of Pu-erh tea. Zhou and Yang (2000)) reported that 20 phenolic compounds together with caffeine were isolated from crude materials of Pu-erh tea produced in Yunnan Province, China. They were identified by spectroscopic method as follows: (−)epicatechin, (−)epigallocatechin, (−)epigallocatechin-3-O-gallate, (−)epicatechin-3-O-gallate, (−)epiafzelechin-3-O-gallate, (+)catechin, (±)gallocatechin, quercetin, quercetin-3-O-β-D-glucopyranoside, rutin, kaempferol, kaempferol-3-O-β-D-glucopyranoside, kaempferol-3-O-rutinoside, strictinin, 1,6-O-digalloyl-β-D-glucopyranose, theogallin, chlorogenic acid, 3-α,5-α-dihydroxy-4-α-cafeoyl-quinic acid, coniferin, gallic acid. Their chemical structures are shown in Fig. 15.5.

Table 15.1 Chemical components of various Chinese sun-dried green teas

Sun-dried green tea	Chemical components (%)										
	TP	TC	FL	TF	TR	TB	TS	TPS	TOS	TE	Ash
Mangjing	35.11	12.03	1.69	0.14	8.68	2.09	11.00	0.11	7.02	39.60	5.05
Nannuo old tea	37.23	11.26	1.26	0.17	6.93	2.20	10.30	0.36	8.55	38.40	4.75
Manggeng old tea	37.80	12.58	1.60	0.14	6.54	2.11	9.50	0.35	6.55	40.00	5.05
Manggeng old tea	36.05	13.09	1.61	0.15	6.88	1.75	10.42	0.90	7.77	40.40	4.75
Manggeng mesa tea	30.97	11.07	1.84	0.23	9.83	2.66	9.26	0.10	6.50	40.20	5.20
Bangwei old tea	29.90	10.21	1.84	0.18	7.61	2.34	9.32	0.15	8.70	37.60	5.25
Bangwei mesa tea	30.62	10.52	1.83	0.21	8.11	2.37	9.21	0.27	5.94	38.60	5.30
Jingmai old tea	35.98	12.80	1.55	0.13	7.92	1.87	9.48	0.21	7.12	39.00	4.75
Jingmai mesa tea	32.36	11.58	1.75	0.18	9.37	3.06	9.22	0.19	8.66	40.00	5.10

Note: *TP* tea polyphenols, *TC* total catechins, *FL* flavone, *TF* theaflavins, *TR* thearubigins, *TB* theabrownins, *TS* total sugar, *TPS* tea polysaccharide, *TOS* tea oligosaccharide, *TE* tea extracts
Source: Gong et al. (2005)

15.3.2 The Changes of Chemical Components of Pu-erh Tea during Solid State Fermentation

15.3.2.1 The Polyphenols

The polyphenols are important active components in tea leaf and account for the 60–80 %, of active ingredients in tea that are responsible for the expression of tea colour, taste and fragrance. The fermentation process of Pu-erh tea with *Aspergillus niger* changes the chemical profile polyphenols (Table 15.2), caffeine, flavone, thearubigins and soluble oligosaccharides decreased during SSF. The theabrownins and soluble tea polysaccharides increase significantly. While the teaflavins, total soluble carbohydrates and ash were not remarkably changed (Tables 15.2 and 15.4). It is thought that these variations during the fermentation process could be considered as being quality characteristics of Pu-erh tea.

Guo et al. (2001) also reported that the catechins ester decreased markedly more than the other catechins. While, gallic acid also increased significantly (Table 15.3) as did the insoluble tea polyphenols by up to 70–80 % which contribute to the reduction of the bitterness of the tea infusion.

Pu-erh tea infusion characteristically has a red to red-brown colour that persists as a consequence of its chemical profile. The theaflavins are responsible for the bright infusion colour while the thearubigins are associated with both colour and

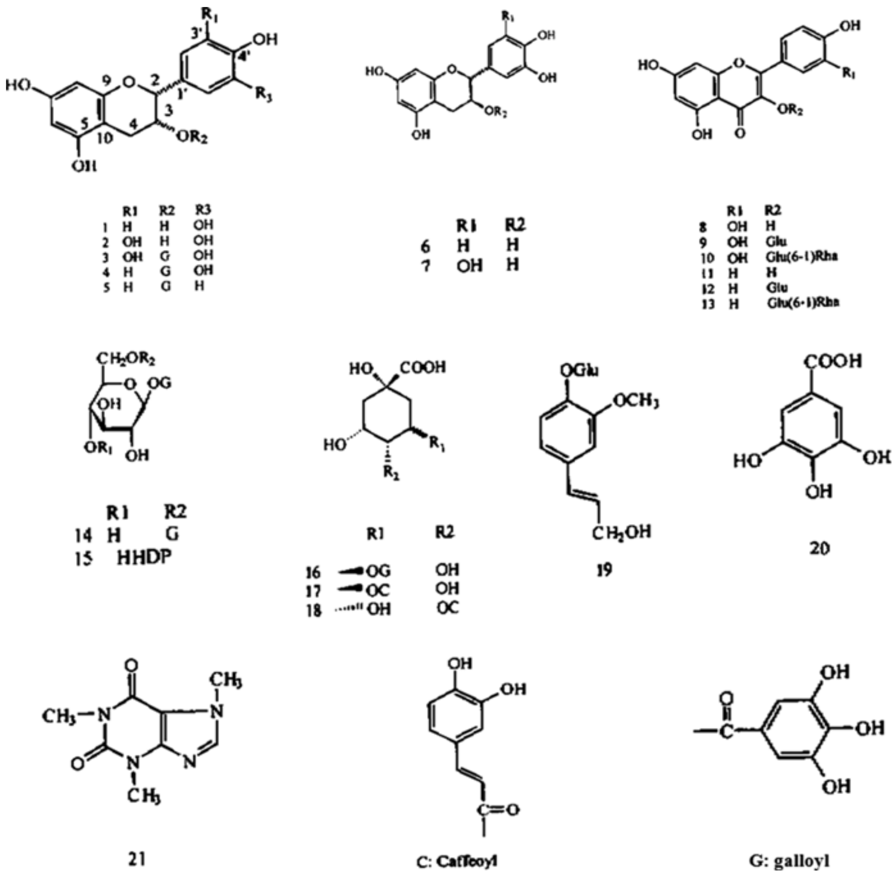

Fig. 15.5 The chemical structures of compounds 1–21. *Source*: Modified from Zhou and Yang (2000)

taste intensity of the infusion. Theabrownins is associated with the darkening of the infusion; when at levels of 6–8 %, the infusion colour appears a bright red-brown, at lower levels (5 %), the infusion colour tends to a bright red-orange colour associated with insufficient fermentation.

In the SSF process, theabrownins levels remain stable while in the other the main chemical constituents of tea are significantly altered (Table 15.2). Moreover, this theabrownins content is considerably high, and considered to be a significant quality attribute of the tea.

Luo et al. (1998) thought that the interactions between polyphenols and production of insoluble protein-polyphenols complex persist and increase as in the tea leaf residues as SSF progresses. Hot and damp conditions that persist inside batches of fermenting tea facilitate the oxidation of tea polyphenols and formation of complex protein-polyphenols complex.

Table 15.2 Chemical components of different upturned tea in the solid state fermentation of Pu-erh tea

Samples and fermentation time	Chemical components (%)					
	TP	TC	FL	TF	TR	TB
Central tea of first pile (10 days)	31.05	9.07	1.46	0.16	4.00	2.35
Mixed tea of first pile (10 days)	33.3	9.84	1.60	0.17	6.41	2.25
Surface tea of second pile (20 days)	24.19	8.12	1.39	0.17	4.61	3.75
Central tea of second pile (20 days)	30.13	7.23	1.58	0.15	6.06	3.23
Lower tea of second pile (20 days)	26.85	7.44	1.42	0.16	5.30	4.14
Cankered bottom tea of second pile (20 days)	26.45	6.64	1.56	0.16	4.8	4.37
Surface tea of third pile (30 days)	18.6	7.73	1.13	0.13	2.34	5.71
Central tea of third pile (30 days)	26.91	5.91	1.42	0.15	4.54	1.93
Bottom tea of third pile (30 days)	26.52	6.4	1.36	0.16	4.76	4.14
Mixed tea of third pile (30 days)	26.52	5.88	1.36	0.16	4.97	4.74
Surface tea of fourth pile (40 days)	12.45	1.97	0.59	0.10	0.28	13.74
Central tea of fourth pile (40 days)	11.18	1.04	0.79	0.11	0.01	11.63
Lower tea of fourth pile (40 days)	13.92	1.90	0.64	0.12	0.26	12.45

Note: *TP* tea polyphenols, *TC* total catechins, *FL* flavone, *TF* theaflavins, *TR* thearubigins, *TB* theabrownins
Source: Gong et al. (2005)

Table 15.3 Content of catechins in different Pu-erh tea (*Unit*: %)

Pu-erh tea	L-EGCG	L-ECG	L-EC	Total catechins	Caffeine	Gallic acid
Yunnan Tuocha	4.65	4.37	trace	9.48	3.82	0.34
Yunnan Pu-erh tea	0.03	0.01	0.21	0.27	3.93	1.72
Yunnan Chitsu Pingcha	0.14	0.19	0.40	0.78	2.82	1.12

Note: *EC* epicatechin, *EGC* epigallocatechin, *EGCG* epigallocatechin gallate
Source: Guo et al. (2001)

The irreversible protein-polyphenols complex is therefore the main reason for the increase of insoluble tea polyphenol content during the SSF process. This is generally caused by quinine (formed from the oxidation of catechins) and the insoluble thearubigins and theabrownins combining with protein. The catechins too, under certain conditions, can also interact with proteins to produce the insoluble complex.

These insoluble protein complexes are reported to be responsible for the reduction of tea extracts from 35.6 to 27 % (Fig. 15.6).

In addition, peroxidase may also promote the oxidation of theaflavins to form insoluble protein complexes. Because the fermentation temperature is quite high approximately 40–60 °C, peroxidase activity reaches optimal levels; as a result, the quantity of theaflavins diminish while that of insoluble thearubigins increase. In conclusion, the production of insoluble protein-polyphenols complex improves the characteristic taste of Pu-erh tea.

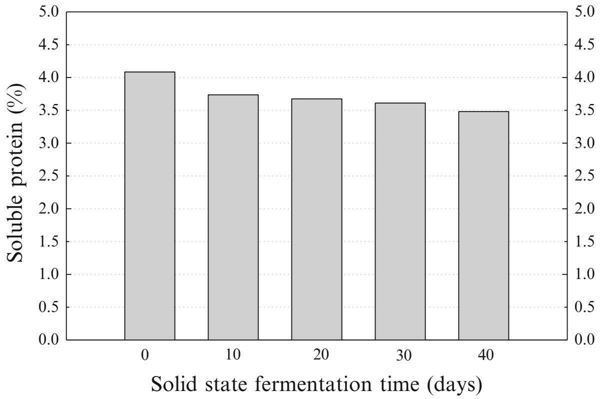

Fig. 15.6 Changing of soluble protein of sun-dried green tea during SSF fermentation. *Source*: Gong et al. (2005)

Table 15.4 Carbohydrates content of different upturned tea during solid state fermentation of Pu-erh tea

	Components (%)				
Tea samples during SSF	TS	TPS	TOS	TE	Ash
Central tea of first pile (10 days)	8.88	0.60	6.51	35.60	5.53
Mixed tea of first pile (10 days)	9.12	0.37	6.51	34.60	5.60
Surface tea of second pile (20 days)	8.31	0.94	6.43	35.53	5.99
Central tea of second pile (20 days)	8.79	1.27	5.49	34.39	5.44
Lower tea of second pile (20 days)	8.39	1.28	4.87	31.40	5.34
Cankered bottom tea of second pile (20 days)	8.79	1.49	4.53	36.67	5.64
Surface tea of third pile (30 days)	9.19	2.25	4.57	33.40	5.53
Central tea of third pile (30 days)	10.02	2.43	5.18	32.89	5.30
Bottom tea of third pile (30 days)	8.72	1.37	4.42	31.82	5.22
Mixed tea of third pile (30 days)	10.24	2.14	4.27	34.93	5.39
Surface tea of fourth pile (40 days)	8.62	3.19	2.18	26.00	5.90
Central tea of fourth pile (40 days)	7.78	3.09	1.66	24.53	5.70
Lower tea of fourth pile (40 days)	8.48	3.79	2.97	27.00	6.12

Note: *TS* total sugar, *TPS* tea polysaccharide, *TOS* tea oligosaccharide, *TE* tea extract
Source: Gong et al. (2005)

15.3.2.2 The Carbohydrates

The tea contains approximately 10–20 % carbohydrates with a profile that is considered to be related to quality. Table 15.4 shows that unfermented Pu-erh tea contains between 9 and 11 % soluble carbohydrates, when fermented by SSF the soluble polysaccharides and oligosaccharides content change.

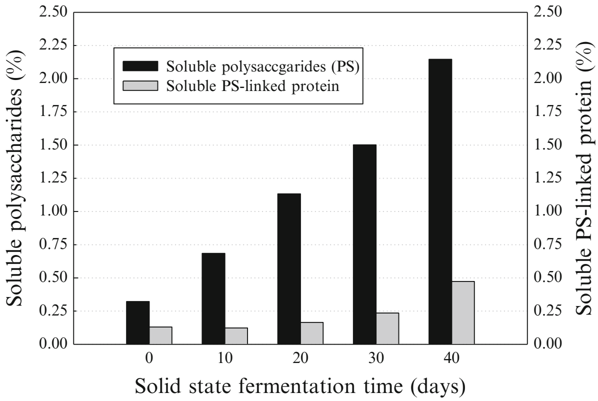

Fig. 15.7 Changing of soluble polysaccharides and polysaccharides-linked protein of sun-dried green tea during SSF fermentation. *Source*: Gong et al. (2005)

The soluble polysaccharides levels change significantly during SSF with the polysaccharide content increasing more than fivefold during SSF (Gong et al. 2005). Moreover, the crude extracts of polysaccharides also contain partially soluble protein (Fig. 15.7) also increase which is consistent with the premise that there is a connection between tea polysaccharides and protein levels.

Luo et al. (1998) indicated the change of soluble carbohydrates content of Pu-erh tea varied during SSF fermentation, after initially falling in concentration the general tendency is for an overall increase in soluble carbohydrate concentration. It has been reported that carbohydrates content decreased because of tea fermentation and storage in a storehouse. For the Yunnan green tea, the content of soluble sugars is 71.10 %, but the soluble sugars in loose Pu-erh tea is 20.3 %, and approximately 36.1 % in Pu-erh Tuocha. The content of soluble sugars being attributed to the growth of microorganisms as soluble sugars can partially serve as the good carbon source for microorganisms' growth.

15.3.2.3 The Changes of Nitrogen Compounds

At the beginning of processing Pu-erh tea, the taste quality is formed by the oxidative degradation of the precursors of the material that will ultimately develop the taste. Caffeine, theabrownins and theine do not vary significantly. Other amino acids such as threonine, glutamic acid and aspartic acid found in fresh leaf tea decreased gradually during SSF, but others such as lysine, phenylalanine, methionine and isoleucine increased during solid state fermentation.

The amino acid profile in the teas are the key factors that determine quality and taste of the infusion. The combination of amino acids and polyphenols provide the characteristics of the tea. Japanese scientists have investigated the composition and contents of amino acids in Pu-erh tea and have shown that every 100 g of Pu-erh tea contained some 16 mg of amino acids. The hydrolysis of protein increased the content of soluble amino acids at the beginning of fermentation time; however, due to microbial growth facilitated by long periods of processing in hot and damp environments, total amino acids decreased. The chemistry involved is thought to involve the partial decomposition of amino acids, oxidation, hydrolysis and deaminizing, and use by microorganisms as nitrogen sources. The SSF process therefore causes shifts in the amino acid profile.

15.3.2.4 Variation of Aromatic Substances

The formation of the characteristic fragrance is especially complicated. It is known that the content of aromatic substances in fresh leaf tea is 0.03–0.05 % that does change during the manufacturing process of Pu-erh tea.

During SSF of Pu-erh tea, many factors such as enzyme catalysis, oxidation and reduction of catechins, moisture and acidic conditions all have the potential to create aromas. From studying Chinese brick tea, Kawakami (1987) identified 132 kinds of fragrance ingredients from brick tea samples. It was thought that this process could be manipulated by controlling microbial fermentation and oxidation to produce desirable aromas.

Chen fragrance components in different raw materials of Pu-erh tea are totally different (Gong et al. 2005). According to Liu and Ina (1987), the main Chen fragrance of Yunnan Pu-erh tea were mainly caused by oxidized linalool (I), oxidized linalool (II), oxidized linalool (IV), linalool, 1,2-dimethoxy-4-ethyl benzene, 1,2-dimethoxy-4-methyl benzene, 1-ethyl-2-formacyl pyrrole, α-terpineol, benzaldehyde, trans-2-trans-4-heptadienal, n-nonanal, n-decanal and other unknown compounds. It is also indicated that the increased contents of octadiene alkone, heptadiene alkone and pentenol were the components that produced Chen fragrance. In addition, the increased fragrant components of 1,2-dimethoxy-4-methyl benzene, 1,2-dimethoxy-4-ethyl benzene and 1,2,3-trimethoxy-4-ethyl benzene of Pu-erh tea resulted from the hydroxylation of gallic acid and the methylation of acyl gallnut.

15.3.2.5 Variation of Tea Extracts

Tea extract is obtained when tea leaves are soaked in hot water; it is the major source of taste and aroma in tea infusions. The high content of tea extract reflects high soluble matter containing tea that to a certain extent is a determinant of the tea quality.

Traditionally processed Pu-erh tea has its unique style. Pu-erh tea is classified as a post-fermented tea and contains soluble carbohydrates and pectin and hydrolysed products that are formed during the fermentation process, which enhance the taste of tea infusion. Shao et al. (1994) reported that the content of tea extracts is very much related to the quality of Yunnan Pu-erh tea. As shown in Table 15.5, the content of tea extracts of Pu-erh tea depends on the solid state fermentation time.

Table 15.5 Variation of tea extracts during solid state fermentation of Pu-erh tea

Tea samples during SSF fermentation	Tea extracts (%)
Central tea of first pile (10 days)	35.60
Mixed tea of first pile (10 days)	34.60
Surface tea of second pile (20 days)	35.53
Central tea of second pile (20 days)	34.39
Lower tea of second pile (20 days)	31.40
Cankered bottom tea of second pile (20 days)	36.67
Surface tea of third pile (30 days)	33.40
Central tea of third pile (30 days)	32.89
Bottom tea of third pile (30 days)	31.82
Mixed tea of third pile (30 days)	34.93
Surface tea of fourth pile (40 days)	26.00
Central tea of fourth pile (40 days)	24.53
Lower tea of fourth pile (40 days)	27.00

Source: Gong et al. (2005)

The longer fermentation time shows the less content of tea extracts of Pu-erh tea. Therefore, during solid state fermentation, the fermentation time is practically a key factor controlling the tea extracts.

15.3.3 The Changes of Chemical Composition of Pu-erh Tea during Storage

Through the analysis of Pu-erh tea that had different storing times and which had been sourced from different producer sources, it was possible to establish that storing time does not play particularly for the physicochemical changes of tea components (Table 15.6). It is clear that the changing components of Pu-erh tea have no significant correlation with the longer storing time; therefore, any changes may be caused by differences in processing methods, raw materials used and storage conditions (Gong et al. 2005). It is therefore quite difficult to determine the age of Pu-erh tea through the changes of chemical components. The means to characterize the age of Pu-erh tea based on scientific means is therefore a challenge.

15.4 Profiles of Microorganisms on the Quality of Pu-erh Tea

Chen et al. (1985) and Hu (1979) independently identified a number of microorganisms, namely *Aspergillus niger*, *Penicillium*, *Rhizopus*, *Aspergillus glaucus*, *Saccharomyces* and *Cladosporium* involved in the processing Pu-erh tea. Among them, *Aspergillus niger* was found in approximately 80 % of the sources tested.

Table 15.6 Chemical components of crusted Pu-erh tea

Yunnan Pu-erh tea samples	Content of component (%)											
	TP	TC	FL	TF	TR	TB	TS	TPS	TOS	TE	Ash	TP
Pu-erh tea (8-class) (1984)	14.34	1.98	3.77	0.27	4.52	9.21	11.15	3.39	3.49	30.51	5.10	14.34
Pu-erh tea 79072 (1989)	12.92	2.04	3.31	0.23	3.44	8.27	9.51	2.64	2.59	24.19	5.80	12.92
Chitsu Pingcha (1997)	12.11	1.35	2.49	0.17	1.98	9.75	8.65	2.63	2.31	36.62	7.16	12.11
Brick tea (1998)	8.31	1.11	1.79	0.18	1.21	11.42	7.74	nil	2.50	31.15	7.58	8.31
Chitsu Pingcha (1999)	12.34	1.48	3.52	0.16	2.42	8.93	8.67	2.61	2.29	36.66	7.09	12.34
Tuocha (2000)	10.62	1.10	1.98	0.15	2.24	10.85	8.21	3.23	1.36	34.50	6.87	10.62
Tuocha (2002)	12.25	1.35	2.52	0.19	2.81	9.53	9.36	3.13	1.49	37.15	6.98	12.25
Pu-erh tea (2003)	12.51	1.10	2.04	0.16	2.24	9.81	9.33	2.38	2.74	32.84	7.10	12.51
Pu-erh tea (2-class) (2003)	10.36	1.07	1.58	0.34	2.24	9.90	8.80	2.59	1.98	22.00	6.25	10.36
Pu-erh tea (4-class) (2003)	10.74	1.51	1.4	0.262	1.71	12.76	9.08	2.77	2.48	25.20	6.40	10.74
Pu-erh tea (7-class) (2003)	13.76	2.21	2.39	0.29	3.36	10.56	10.16	3.39	3.11	27.87	5.40	13.76
Pu-erh tea (8-class) (2003)	16.14	2.81	2.22	0.32	4.55	9.13	11.32	3.47	3.91	30.13	5.45	16.14
Old Tuocha (2004)	15.54	3.52	3.87	0.16	1.37	12.25	10.78	0.31	3.26	35.20	5.95	15.54

Note: *TP* tea polyphenols, *TC* total catechins, *FL* flavone, *TF* theaflavins, *TR* thearubigins, *TB* theabrownins, *TS* total sugar, *TPS* tea polysaccharide, *TOS* tea oligosaccharide, *TE* tea extract
Source: Gong et al. (2005)

Observations of SSF revealed that in the early phases of SSF rapid growth and propagation of mesophilic moulds such as *Aspergillus niger* was observed. However, later the SSF process, under dry environments psychrophiles such as *Aspergillus glaucus* started to grow. At the same time, various enzymes underwent reactivation that then caused further chemical changes in the tea composition profiles (Hu 1957, 1979).

Other recent studies by Zhou and Zhao on microbes during the solid state fermentation process of Yunnan Pu-erh tea showed that in addition to the Microorganisms identified independently by Hu and Chen other Aspergilli namely *Aspergillus terreus*, *Aspergillus candidus* and other bacteria were also significant microbes of Pu-erh tea. By far *Aspergillus niger* was the largest contributor. Another significant microorganism identified was *Saccharomyces* sp. These microbes all play direct and indirect roles more or less on quality formation of Pu-erh tea (Zhou et al. 2004; Zhao and Zhuo 2005).

15.4.1 Aspergillus niger

Aspergillus niger is a generally significant industrial microorganism and specifically has been identified as a key organism in the production of Pu-erh tea. Its growth is beneficial to the quality improvement of Pu-erh tea. *Aspergillus niger* belongs to *Deuteromycotina*, *Hyphomycetes, Moniliales, Moniliaceae* and *Aspergillus*. During solid state fermentation of Pu-erh tea, *Aspergillus niger* may secrete some twenty (20) types of hydrolytic enzymes (Zhao and Zhou 2003). Among them, glucoamylase, cellulases and pectinase decompose many insoluble organic compounds such as polysaccharides, fat, protein, natural fibre and pectin.

After hydrolysis of macromolecular compounds, the micromolecular compounds produced are monosaccharides, amino acids, hydrated pectin and soluble carbohydrates. The organic acids produced by enzymatic activity may promote the development of the desired taste such as the mellow quality characteristics of tea infusion.

15.4.2 Penicillium

Penicillium belongs to *Deuteromycotina, Hyphomycetes, Moniliales* and *Moniliaceae. Penicillium,* isolated from Pu-erh tea, can produce a number of enzymes such as glucose oxidase and secrete organic acids including gluconic acid, citric acid and antiscorbutic acid. It also may produce penicillin to eliminate and suppress the growth of putrefactive bacteria; therefore, *Penicillium* is considered to improve the tea quality.

15.4.3 Rhizopus

Rhizopus belongs to *Rhizopus, Zygomycotina, Zygomycetes, Mucorales* and *Mucoraceae*. It can be used to ferment Pu-erh tea (Liu and Ina 1987). It can also produce a number of enzymes such as amylase, sugar forming enzymes, pectinase, proteinase and zymase. These enzymes are active at temperatures ranging from 32 to 40 °C and pH 4.5–6. Among them, the activity of amylase is higher than those of other enzymes. Amylase secreted by *Rhizopus* can catalyse starch in the Pu-erh tea and produce organic acids such as fumaric acid, lactic acid and succinic acid. *Rhizopus* can also produce fragrant ester matters, by transforming sterol race compounds to their derivatives. So, *Rhizopus* is an important organism for Pu-erh tea. In addition, because of its pectinase activity, *Rhizopus* can break down tea leaves during solid state fermentation. Therefore, during solid state fermentation, by controlling suitable temperature and humidity for the growth of *Rhizopus*, this will be beneficial to form the sweet and mellow taste of Pu-erh tea.

15.4.4 Saccharomyces *Yeast*

The *Saccharomyces* yeast belongs to the *Ascomycetes*, *Hemiascomycetes*, *Endonycetales* and *Saccharomyces*. It is an important strain which is also responsible for forming the quality of Pu-erh tea. In the fermentation process of Pu-erh tea, the function of hot and damp provides suitable environment for *Saccharomyces* growth and its metabolic activity. This also strengthens the activities of enzymes which result in the changes of tea composition.

In addition, many of the monosaccharides and soluble oligosaccharides produced by *Aspergillus niger* also can be supplied as carbon source for the growth of this yeast. It was indicated that provision of suitable conditions for rapid *Saccharomyces* growth improved the quality of Pu-erh tea with sweet and mellow characteristics (Gong et al. 2005).

Interestingly, Pu-erh tea with the quality characteristic of Chen fragrance (mellow and sweet) is related to the rapid growth of *Saccharomyces* in solid state fermentation of Pu-erh tea. Therefore, appropriate control in the quantity of *Saccharomyces* in processing Pu-erh tea may result in the increasing of those effective nutrients and healthy components of Pu-erh tea, which provide the unique qualities and style of Pu-erh tea. On the other hand, if such control is not appropriate, there could be deterioration of taste and therefore quality of Pu-erh tea.

15.4.5 *Bacteria*

In a mixed matrix, bacterial growth dominates in conditions of higher temperature and humidity together with a rich source of organic material. Such conditions will produce off-odours and or a rancid taste. However, during the processing of Pu-erh tea, bacterial populations are particularly low and so far the presence of pathogenic bacteria has not been reported. This scenario might be due to growth competition among microorganisms and repression of bacteria by the tea polyphenols.

15.5 Research on Safety of Pu-erh Tea

Pu-erh tea, as a traditional beverage of China, has a long history of culture. They have been drinking Pu-erh tea without particular known risk. However, in a view of the fact that the processing of Pu-erh tea deals with a number of microorganisms a significant number of microbial metabolites and complex compounds are produced. The potential for health hazards is not entirely unfounded; therefore, the chemical toxicity of Pu-erh tea has been evaluated and reports show that in brick format there is ample proof of safety (Liu et al. 1996; Chou et al. 1999; Cao et al. 1998, 2001, 2003; Wong et al. 2003).

Liu et al. (2003), at Southwest Agricultural University, studied on acute toxicity of three typical Pu-erh teas and one baked green tea from Yunnan Province and concluded that all four kinds of tea are highly safe for drinking.

Sun and Liu (2002), from Taiwan University reported that *Aspergillus* aflatoxin was not separated from Pu-erh tea inoculated with aflatoxin-producing *Aspergillus* during simulated production; it seemed that aflatoxin-producing *Aspergillus* could not survive under microflora system of Pu-erh tea fermentation, where *Aspergillus niger* and *Saccharomyces*, the dominant microorganisms, suppressed the growth of harmful microorganisms.

References

Cao J, Zhao Y, Liu JW (1998) Safety evaluation and fluorine concentration of Pu’er brick tea and Bianxiao brick tea. Food Chem Toxicol 36(12):1061–1063

Cao J, Zhao Y, Liu JW (2001) Processing procedures of brick tea and their influence on fluorine content. Food Chem Toxicol 39(9):959–962

Cao J, Zhao Y, Liu JW, Xirao R, Danzeng S, Daji D, Yan Y (2003) Brick tea fluoride as a main source of adult fluorosis. Food Chem Toxicol 41(4):535–542

Chen ZD, Liu QJ, Zhou CQ (1985) The microorganism and the Pu-erh tea ferment. J Tea Sci Technol 4:4–7, In Chinese

Chen YS, Liu BL, Chang YN (2010) Bioactivities and sensory evaluation of Pu-erh teas made from three tea leaves in an improved pile fermentation process. J Biosci Bioeng 109(6):557–563

Chou CC, Lin LL, Chung KT (1999) Antimicrobial activity of tea as affected by the degree of fermentation and manufacturing season. Int J Food Microbiol 48(2):125–130

Gong JS, Zhou HJ, Zhang XF, Song S, An WJ (2005) Changes of chemical components in Pu’er tea produced by solid state fermentation of sundried green tea. J Tea Sci 25(3):126–132

Guo WF, Wu D, Sakata K, Luo SJ, Yang XF (2001) Proceedings of 2001 international conference on O-CHA [tea] culture and science (Session II), 5–8 Oct, Shizuoka, Japan, pp 288–291

Hu JC (1957) Separation and identification of microorganism from four kinds of Bianxiao tea. J Tea 2:20–22, In Chinese

Hu JC (1979) Study on the *Aspergillus* of Pu-erh tea. J Tea 2:20–22, In Chinese
Kawakami B (1987) Aroma characterization for pile tea, Chinese brick tea and black tea. Nihonnokeikagakukaishi 61(4):457–465, In Japanese
Liu QJ, Ina K (1987) Study on the aroma components of Pu-erh tea. J Southwest Agric Univ 9(4):470–475, In Chinese
Liu CH, Hsu WH, Lee FL, Liao CC (1996) The isolation and identification of microbes from a fermented tea beverage, Haipao, and their interactions during Haipao fermentation. Food Microbiol 13(6):407–415
Liu QJ, Chen WP, Bai WX, Li QZ (2003) Acute toxicity evaluation of Pu-erh tea. J Tea Sci 23(2):141–145, In Chinese
Luo LX, Wu XC, Deng YL, Fu SW (1998) Variations of main biochemical components and their relations to quality formation during pile-fermentation process of Yunnan Pu-erh tea. J Tea Sci 18(1):53–60, In Chinese
Lv HP, Zhang YJ, Lin Z, Liang YR (2013) Processing and chemical constituents of Pu-erh tea: a review. Food Res Int 53(2):608–618
Shao WF, Cai X, Yang SR, Yuan W (1994) A preliminary study on the relation between the contents of chemical constituents and quality of Yunnan Pu'er tea. J Yunnan Agric Univ 9(1):17–22, In Chinese
Sun LX, Liu HQ (2002) Anti- arteriosclerosis function of Pu-erh tea. In: 2002 Year China Pu-erh Tea International Conference. Yunnan People's Publishing Press, pp 309–317
Wong MH, Fung KF, Carr HP (2003) Aluminium and fluoride contents of tea, with emphasis on brick tea and their health implications. Toxicol Lett 137(1–2):111–120
Zhang DY, Shi ZP, Liu YL (2005) Development of pharmacological function of Pu-erh tea. Fujian Chaye 1:12–13, In Chinese
Zhao LF, Zhou HJ (2003) Preliminary study on function of yeast in developing Pu-erh tea quality. Tea Garden 2:4–6, In Chinese
Zhao LF, Zhuo HJ (2005) Study on the main microbes of Yunnan Pu-erh tea during pile-fermentation process. J Shandqiu Teach Coll 21(2):129–133, In Chinese
Zhou ZH, Yang CR (2000) Chemical constituents of crude green tea, the material of Pu-erh tea in Yunnan. Acta Botanica Yunnanica 22(3):343–350, In Chinese
Zhou HJ, Li JH, Zhao LF, Hart J, Yang XJ (2004) Study on main microbes on quality formation of Yunnan Pu-erh tea during Pile-fermentation process. J Tea Sci 24(3):212–218, In Chinese