

# Advances in the chemical composition and fermentation control of dark tea

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## Abstract

Dark tea is a unique type of post-fermented tea that has gained popularity among consumers due to its distinctive flavor profile and potential health benefits. Microbial fermentation is a critical process in the production of dark tea, playing a decisive role in shaping its unique chemical composition, flavor characteristics, and bioactivity. This review systematically summarizes recent advances in research on the distinctive chemical constituents of dark tea, with a particular focus on cutting-edge fermentation control strategies. These strategies include the optimization of environmental parameters and the targeted application of functional microbial strains. By reviewing current research findings and practical techniques, this paper aims to provide a theoretical foundation and technical support for enhancing the production potential and quality of dark tea.

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## Introduction

Dark tea is a type of post-fermented tea and one of the six traditional categories of Chinese tea. Originating in the Northern Song Dynasty<sup>[1]</sup>, it has been widely distributed throughout history as a 'border-sale tea' due to its unique flavor and health benefits<sup>[2]</sup>. Through both land and maritime trade routes<sup>[2,3]</sup>, dark tea gradually spread to the rest of the world (Fig. 1a). In recent years, with the growing global interest in health-promoting beverages, dark tea has attracted increasing attention and demand in the international market, owing to its potential effects in aiding digestion, reducing blood lipids, and controlling body weight.

In the production of dark tea, the fresh leaves must first undergo preliminary processing to produce raw dark tea, which serves as the fundamental raw material for the subsequent manufacturing of various dark tea products. The processing of raw dark tea typically involves four basic steps: fixing, rolling, pile fermentation, and drying<sup>[4]</sup> (Fig. 1b). Once raw dark tea is prepared, it can be further processed into a wide range of dark tea products depending on the specific techniques applied. Based on the final product form, dark tea can be broadly classified into two major categories: loose-leaf tea and compressed tea. Loose-leaf tea is directly packaged after grading, screening, and drying of raw dark tea, with representative varieties including Tianjian tea, Gongjian tea, and Shengjian tea. Compressed tea refers to raw dark tea that is steamed and softened before being molded into various forms. Some types may also undergo subsequent post-fermentation to further develop their unique characteristics. Common forms include brick tea, cake tea, tuo cha, huajuan tea, and qianliang tea. Among them, brick tea encompasses a wide variety, such as Fuzhuan brick tea (FZT), Qingzhuan tea (QZT), Liubao tea (LBT), Heizhuan brick tea, and Tibetan tea (TT).

Cake and tuo teas are generally round in shape, with Pu-erh tea (PET) being the most representative of cake teas. Huajuan and Qianliang teas are typically cylindrical in form<sup>[5]</sup> (Fig. 1c).

Microbial fermentation plays a particularly critical role in the processing of dark tea and is a key factor in determining its characteristic quality. During this stage, tea leaves are piled under controlled environmental conditions with specific temperature and humidity levels. Under the synergistic effects of endogenous enzymes and microbial metabolism, a series of unique chemical transformations occur, leading to the initial formation of the distinctive qualities of dark tea. This process also results in the production of numerous novel compounds, such as Fuzhuanin A–F, teadenol A and B (catechin A- and B-ring cleavage products), C-glycosylated flavonols, and flavan alkaloids<sup>[6]</sup>.

In terms of fermentation process optimization, recent advancements in dark tea production have increasingly focused on two key aspects: process parameter control and microbial intervention. On one hand, regulating critical parameters such as temperature, humidity, fermentation duration, and pile thickness can effectively influence the structure and metabolic activity of microbial communities, thereby modulating the formation of key flavor compounds and bioactive constituents in the tea. On the other hand, the selection and targeted inoculation of specific functional microbial strains have emerged as important strategies for enhancing fermentation efficiency and improving product quality. This approach involves the artificial construction of dominant microbial consortia to create favorable conditions for desired metabolic pathways, thereby promoting the enrichment of functional components. These regulatory strategies not only improve traditional processing methods but also provide a scientific foundation for enhancing the quality and functionality of dark tea products.



**Fig. 1** The overview of historical routes, manufacturing, and morphological types of dark tea. (a) Dark tea trade routes: overland (orange) transported to Asia and Europe; maritime (blue) transported to Southeast Asia, Europe, and the Americas. (b) Traditional and modern processes of dark tea production. (c) Morphological types of dark tea: loose-leaf and compressed.

This review provides a comprehensive summary of the characteristic chemical constituents of dark tea and the regulatory technologies influencing their formation. It explores the key compounds affecting tea quality, the processing conditions involved, and the underlying mechanisms of compositional transformation, aiming to offer a theoretical foundation for future advancements in dark tea processing and quality improvement.

## Chemical constituents

To date, over 1,000 non-volatile compounds have been isolated and identified from tea leaves. The main functional constituents include catechins and their polymers, flavonoids, phenolic acids, alkaloids, terpenoids, and others<sup>[6]</sup>. The functional components in tea are derived both from compounds formed during the metabolic processes of the tea plant's growth and from new compounds generated through the transformation of chemical substances in fresh tea leaves during processing. In the production of dark tea, under the influence of microbial enzymes and moist heat, the components of the tea undergo hydrolysis, oxidation, and polymerization reactions, leading to the formation of multiple new compounds (Supplementary Table S1). Moreover, the contents of chemical components vary significantly among different types of dark tea (Table 1)<sup>[7,8]</sup>.

## Catechins and their polymers

Tea leaves are rich in polyphenolic compounds, which account for 20%–35% of the dry weight of tea. Catechins, the main components of tea polyphenols, make up approximately 60%–80% of the total polyphenolic compounds. These compounds possess a flavan-3-ol structure and include eight primary types: EGCG (1), EGC (2), ECG (3), EC (4), GCG (5), GC (6), CG (7), and C (8)<sup>[9]</sup>.

Notably, in contrast to other tea components, dark tea undergoes natural oxidation, polymerization, and microbial hydrolysis and cleavage, leading to the formation of numerous catechin derivatives, including oxidation products and ring-cleavage products.

The catechin-derived polymers in dark tea primarily consist of theaflavins (TFs), thearubigins (TRs), and theabrownins (TBs). TFs (0.1%–0.2%) and TRs (1%) are present in relatively low concentrations in dark tea, whereas TBs are more abundant, ranging from 7% to 13%. TBs are the most abundant and biologically active pigments in dark tea<sup>[10]</sup>. TFs and TRs are formed by the polymerization of catechins under the catalytic action of polyphenol oxidase. To date, over 20 different TF compounds have been isolated from tea, including TF, theaflavin-3-gallate (TF-3-G), and theaflavin-3,3-digallate (TF-3,3-G). TRs are a complex, heterogeneous group of phenolic compounds. Compared to catechins and TFs, TRs have a higher degree of polymerization, larger molecular weight, stronger metal ion chelation ability, and greater resistance to enzymatic hydrolysis and glycosylation. During fermentation, catechins are oxidized into TFs

**Table 1.** Dynamic changes of major chemical components during dark tea processing (mg/g).

| Tea type          | PET         | FZT         | LBT         | QZT          | TT           |
|-------------------|-------------|-------------|-------------|--------------|--------------|
| Total polyphenols | 101.6 ± 4.2 | 115.8 ± 4.8 | 166.8 ± 6.9 | 194.2 ± 8.0  | 56.82 ± 0.19 |
| Catechins         | 5.3 ± 0.2   | 52.8 ± 2.4  | 69.6 ± 3.1  | 74.1 ± 3.1   | 6.04 ± 0.13  |
| Theabrownins      | 118.2 ± 4.9 | 36.0 ± 1.5  | 28.7 ± 1.2  | 26.7 ± 1.1   | 56.07 ± 1.3  |
| EGCG              | ND          | 9.3 ± 0.4   | 18.9 ± 0.8  | 19.3 ± 0.8   | ND           |
| EC                | 1.1 ± 0.0   | 5.0 ± 0.2   | 8.0 ± 0.3   | 9.6 ± 0.4    | 2.15 ± 0.06  |
| GCG               | 0.2 ± 0.0   | 0.2 ± 0.0   | 3.1 ± 0.1   | 2.5 ± 0.1    | 0.73 ± 0.03  |
| ECG               | ND          | 1.4 ± 0.1   | 10.0 ± 0.4  | 9.7 ± 0.4    | ND           |
| EGC               | ND          | 34.1 ± 1.4  | 10.5 ± 0.4  | 13.7 ± 0.6   | 2.83 ± 0.01  |
| C                 | 1.1 ± 0.0   | ND          | 13.1 ± 0.5  | 15.3 ± 0.6   | 0.33 ± 0.05  |
| Gallic acid       | 19.3 ± 0.8  | 9.8 ± 0.4   | 14.9 ± 0.6  | 28.8 ± 1.2   | 7.25 ± 0.4   |
| Caffeine          | 38.0 ± 1.6  | 26.0 ± 1.1  | 34.6 ± 1.4  | 34.2 ± 1.4   | 26.08 ± 0.81 |
| Amino acids       | 2.26 ± 0.28 | 3.32 ± 0.42 | 7.07 ± 0.48 | 11.34 ± 0.05 | 14.07 ± 0.23 |

ND, not detected; PET, Pu-erh tea; FZT, Fuzhuan brick tea; LBT, Liubao tea; QZT, Qingzhuana tea; TT, Tibetan tea.

and TRs, which then undergo further oxidation and polymerization to form TBs<sup>[11]</sup>. TBs are even more complex, high-molecular-weight compounds that consist of polymerized phenolic compounds rich in hydroxyl and carboxyl groups<sup>[12]</sup>. They exhibit characteristics typical of phenolic substances. During the fermentation of dark tea, the content of TBs increases significantly with prolonged fermentation time, while the levels of catechins, TFs, and TRs decrease markedly<sup>[13]</sup>. The TB in TT exhibits higher levels of organic acids, lipids, and alcohols in its pyrolysis products compared to other teas, such as PET or FZT, but lower levels of phenolic and ketone compounds. Additionally, the TB in TT demonstrates unique bioactivity in regulating inflammation and oxidative stress, particularly through the Nrf2/NF- $\kappa$ B signaling pathway and microbial metabolites<sup>[14]</sup>.

In addition, under the catalytic action of microbial enzymes, catechin compounds in dark tea undergo hydrolysis, oxidation, and ring cleavage, leading to the formation of numerous new compounds. Recent studies have identified a series of flavan-3-ol A-ring derivatives in ripe Pu-erh tea (RPT)<sup>[15]</sup>, including 8-C-substituted flavan-3-ol catechins (puerins A [9] and puerins B [10])<sup>[16]</sup>, four phenylpropanoid-substituted flavan-3-ol compounds (puerins C–F [11–14])<sup>[17]</sup>, and 8-N-ethyl-2-pyrrolidone-substituted flavan-3-ol catechins (puerins I–VIII [15–22])<sup>[18]</sup>. Tian et al.<sup>[18]</sup> also isolated four new compounds from RPT: 8-carboxymethyl-(+)-catechin (23), 8-carboxymethyl-(+)-catechin methyl ester (24), 6-carboxymethyl-(+)-catechin (25), and 6-carboxyl-(–)-gallocatechin (26), along with three known catechin derivatives: 8-carboxyl-(+)-catechin (27), (+)-catechin-8-C- $\beta$ -D-glucopyranoside (28), and (–)-epicatechin-8-C- $\beta$ -D-glucopyranoside (29). Additionally, compounds such as epicatechin-4R-(4-hydroxyphenyl)-dihydro-2(3H)-pyranone (30) and Cinchonain Ib (31) were also identified<sup>[18]</sup>.

Currently, the new catechin-related compounds found in RPT are primarily flavan-3-ol A-ring cleavage products, while other types of dark tea mainly contain catechin derivatives resulting from flavan-3-ol B-ring cleavage. During the fermentation of FZT, catechins degrade into B-ring cleavage metabolites of catechins, including Fuzhuanins A–F (32–37), planchol A (38), and xanthocerin (39)<sup>[19]</sup>. Notably, two new B-ring cleavage derivatives were isolated through bacterial or fungal biotransformation of flavan-3-ols: (2S)-4-([2R,3S]-3,4-dihydro-3,5,7-trihydroxy-2H-1-benzopyran-2-yl)-3,6-dihydro-6-oxo-2H-pyran-2-carboxylic acid (40) and (2S)-4-([2R,3S]-3,4-dihydro-3,5,7-trihydroxy-2H-1-benzopyran-2-yl)-3,6-dihydro-6-oxo-2H-pyran-2-carboxylic acid (41)<sup>[20]</sup>. Moreover, three B-ring cleavage products of flavan-3-ols, namely teadenol A (42), teadenol B (43), and teasperin (44), were isolated from Yamabukinadeshiko and Kippuku-cha

teas in Japan<sup>[20]</sup>. Two flavan-3-ol derivatives were also identified from Hunan brick tea and LBT: (R)-6-oxo-4-([2R,3R]-3,5,7-trihydroxychroman-2-yl)-3,6-dihydro-2H-pyran-2-carboxylic acid (45)<sup>[21]</sup> and teasperol (46)<sup>[22]</sup> (Fig. 2).

## Flavonoids and flavonoid glycosides

Tea contains various flavonoids, primarily including kaempferol (47), quercetin (48), and myricetin (49)<sup>[23]</sup>. In recent years, researchers have isolated several other aglycones from dark tea, such as luteolin (50), 3',4',5-trihydroxy-7-methoxyflavone (51), and 3',4',7-trihydroxy-5-methoxyflavone (52)<sup>[24]</sup>. In dark tea, flavonoids typically exist in glycoside form, including O-glycosides and C-glycosides. Recent studies have isolated multiple C-glycoside flavonoids from RPT and FZT, such as vicenin-2 (53), isoschaftoside (54), vitexin-2"- $\alpha$ -L-rhamnopyranoside (55), isoorientin (56), 2"-O- $\beta$ -D-glucopyranosylvitexin (57)<sup>[18]</sup>, isovitexin (58), vitexin (59)<sup>[25]</sup>, chafurosides A (60), chafurosides B (61), and vitexin-2"-O-rhamnoside (62)<sup>[26]</sup>.

The main flavonoid glycosides found in RPT include rutin (63), kaempferol-3-O- $\beta$ -D-glucoside (64), kaempferol-3-O- $\alpha$ -L-rhamnopyranosyl-(1-6)- $\beta$ -D-glucopyranoside (65), apigenin-6-C- $\alpha$ -L-arabinopyranosyl-8-C- $\beta$ -D-glucopyranoside (66), vitexin-4"-O- $\beta$ -D-glucoside (67), myricetin-3-O- $\beta$ -D-glucoside (68), quercetin-3-O- $\alpha$ -L-rhamnoside (69), and quercetin-3-O- $\alpha$ -L-rhamnopyranosyl-(1-6)- $\beta$ -D-glucopyranoside (70)<sup>[18]</sup>. In addition, compounds such as quercetin-3-O-glucoside (71)<sup>[18]</sup>, kaempferol-3-O-rutinoside (72), and 3,4-dihydroxy-5-methoxyflavone-7-O- $\beta$ -D-glucopyranoside (73) have also been isolated from RPT<sup>[27]</sup>. Zhe et al.<sup>[28]</sup> isolated Astragalins (74) from RPT. Additionally, they also isolated methylenebisnicotiflorin (75) from RPT<sup>[29]</sup>, a compound rarely found in nature<sup>[30]</sup>. It is the first dimeric polyphenol discovered in dark tea.

FZT contains a wide variety of flavonoids, including vitamin H (76), quercetin-3-O-robinobioside (77), kaempferol-3-O- $\beta$ -D-glucopyranosyl-(1-3)- $\alpha$ -L-rhamnopyranosyl-(1-6)- $\beta$ -D-glucopyranoside (78), and quercetin-3-O- $\beta$ -D-glucopyranosyl-(1-3)-L-rhamnopyranosyl-(1-6)- $\beta$ -D-glucopyranoside (79)<sup>[19]</sup>. Other flavonoids include nicotiflorin (80), rutin (63), myricetin-3-O-rutinoside (81), apigenin 6,8-di-C- $\beta$ -D-glucopyranoside (82), apigenin-6-C- $\alpha$ -L-arabinopyranosyl-8-C- $\beta$ -D-glucoside (83), apigenin-7-O- $\beta$ -D-galactopyranosyl-8-C- $\beta$ -D-glucoside (84), apigenin-7-O- $\alpha$ -L-rhamnopyranosyl-8-C- $\beta$ -D-galactopyranoside (85), isovitexin (58), vitexin (59), myricetin-3-O- $\beta$ -D-glucoside (86), quercetin-3-O- $\beta$ -D-glucoside (87), and kaempferol-3-O- $\beta$ -D-galactoside (88)<sup>[26]</sup>. FZT also contains various acylated glycoside flavonoids, including four kaempferol

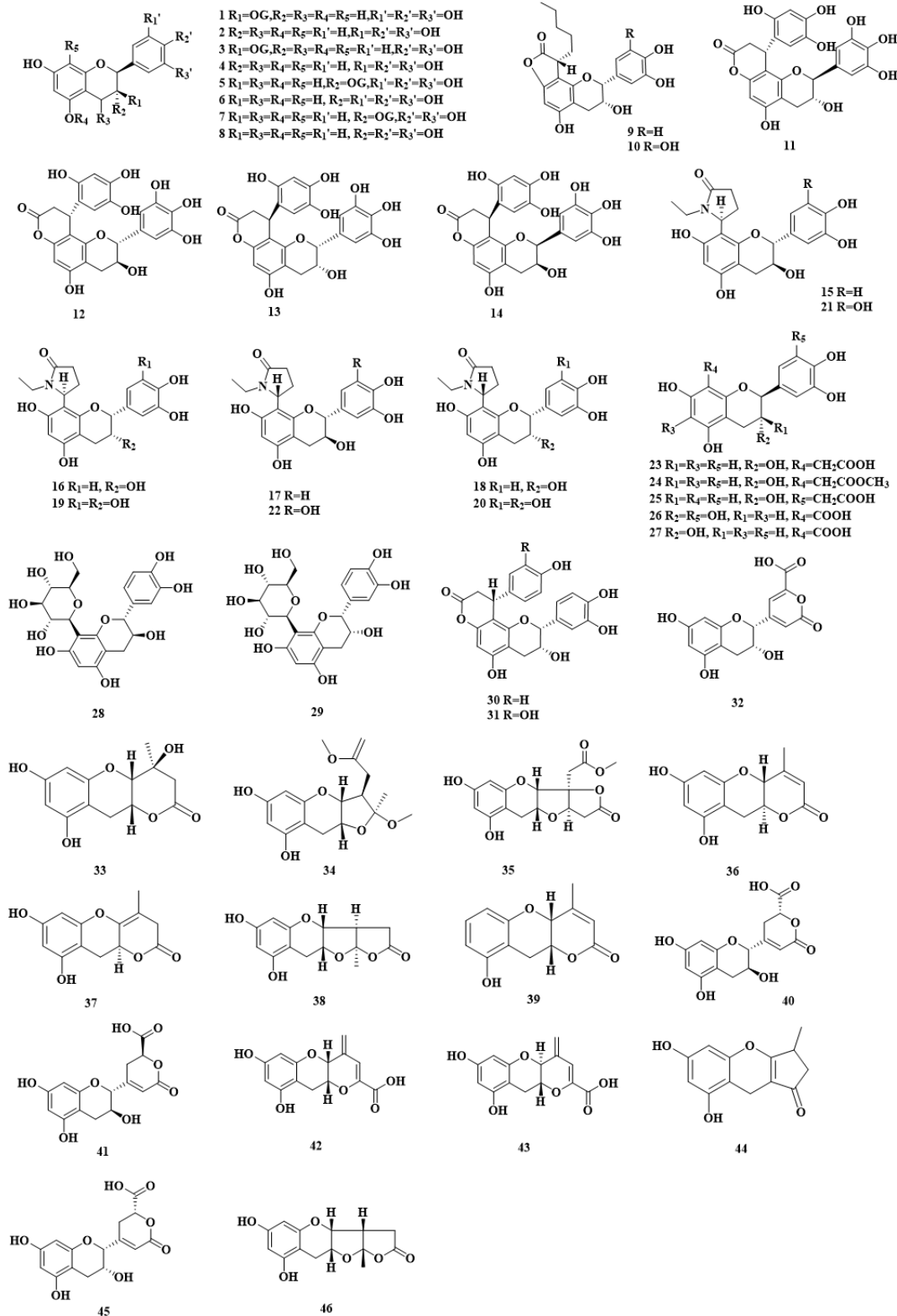


Fig. 2 Chemical structural formulas of catechins and their polymers.

acylglycosides: camellikaempferside A (89)<sup>[31]</sup>, camellikaempferside B (90)<sup>[32]</sup>, camellikaempferside D (91), and camellikaempferside E (92)<sup>[33]</sup>, as well as three quercetin acylglycosides: camelliquercetiside C (93)<sup>[31]</sup>, camelliquercetiside E (94), and camelliquercetiside F (95)<sup>[33]</sup> (Fig. 3).

## Phenolic acids

Tea contains a variety of phenolic acids, including gallic acid (96), caffeic acid (97), ferulic acid (98), salicylic acid (99)<sup>[34]</sup>, with gallic acid being the most abundant. During the processing of dark tea, numerous phenolic acids and their derivatives are formed as degradation products, such as protocatechuic acid (100), succinic acid (101),

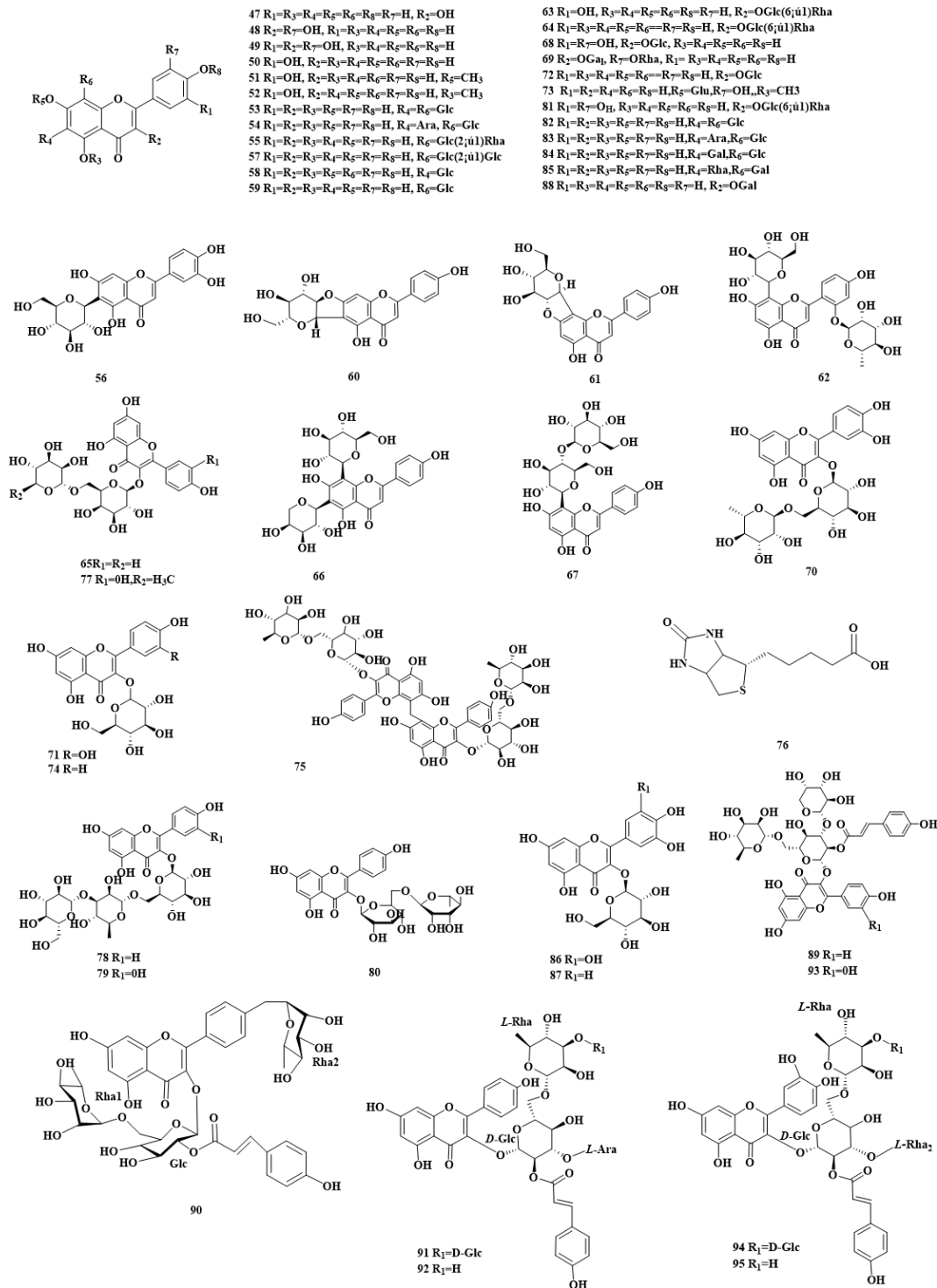
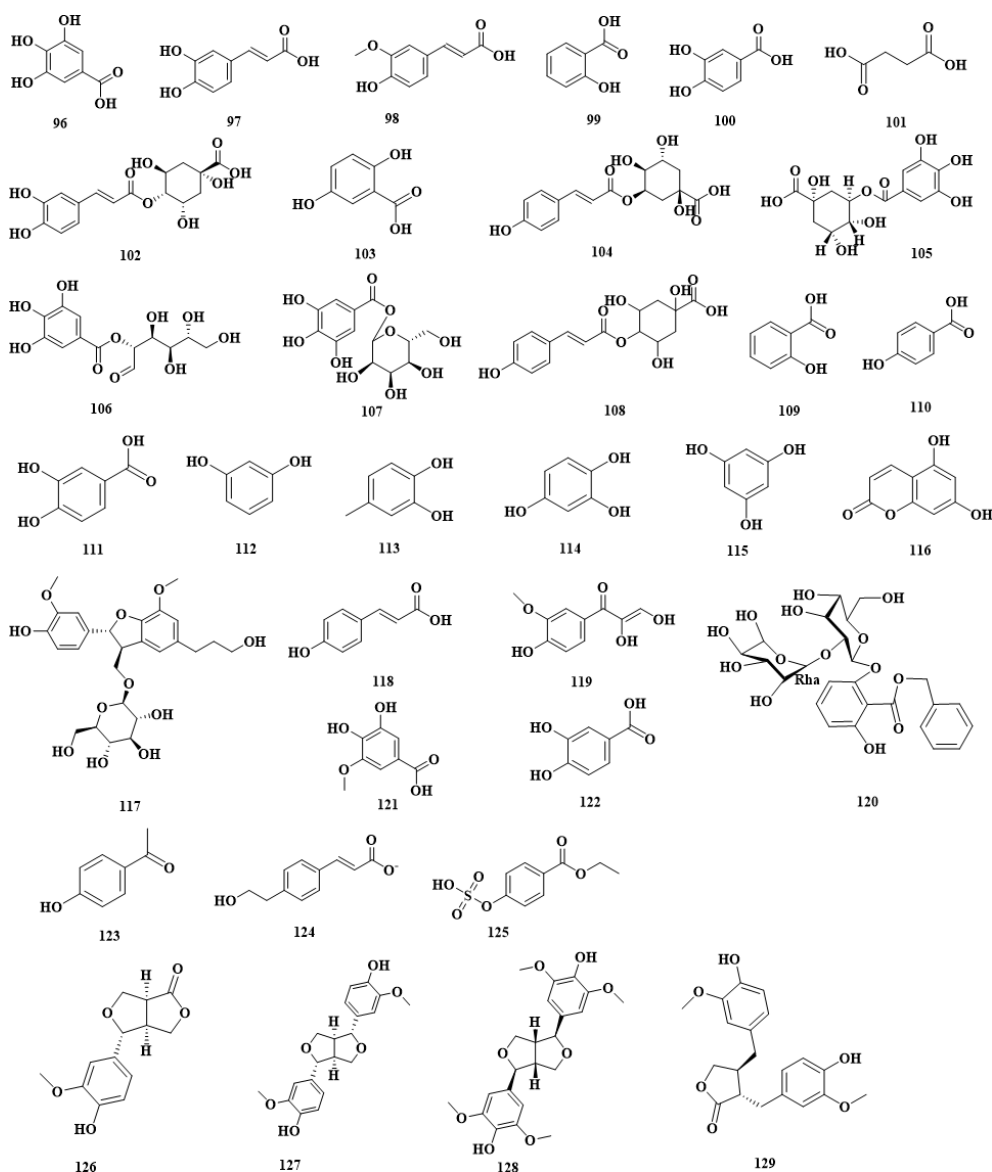


Fig. 3 Chemical structural formulas of flavonoids and flavonoid glycosides.

cryptochlorogenic acid (102), 2,5-dihydroxybenzoic acid (103), and 3-*O-p*-coumaroyl quinic acid (104)<sup>[7]</sup>. Additionally, several phenolic acid derivatives have been isolated from dark tea, including 3-*O*-galloylquinic acid (105), 2-*O*-galloyl-D-glucose (106), 1-*O*-galloyl-β-D-glucopyranose (107)<sup>[18]</sup>, and 4-*O-p*-trans-coumaroyl quinic acid (108)<sup>[29]</sup>.

Studies have found that RPT also contains various phenolic acids and their derivatives, such as 2-hydroxybenzoic acid (109), 4-hydroxybenzoic acid (110), 3,4-dihydroxybenzoic acid (111), 1,3-dihydroxybenzene (112), 4-methyl-1,2-benzenediol (113), and 1,2,4-

benzenetriol (114)<sup>[29]</sup>. In FZT, the content of simple phenolic acids is relatively high, including phloroglucinol (115), 5,7-dihydroxycoumarin (116), (7R,8S)-dihydrodehydrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside (117), *p*-coumaric acid (118), 2,3-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-propen-1-one (119), benzyl-2-neohesperidoside-6-hydroxybenzoic acid (120)<sup>[26]</sup>, 3-methoxy-4,5-dihydroxybenzoic acid (121), and 3,4-dihydroxybenzoic acid (122)<sup>[35]</sup>. Additionally, several compounds have been isolated from Qianliang tea, including *p*-hydroxyacetophenone (123), *p*-hydroxyethyl cinnamate (124), ethyl 4-(sulfoxy)benzoate (125),



**Fig. 4** Chemical structural formulas of phenolic acids.

salicifoliol (126), (-)-pinoresinol (127), (+)-lirioresinol-A (128), and (+)-matairesinol (129)<sup>[20]</sup> (Fig. 4).

## Alkaloids

Tea is one of the plants with a relatively high content of purine alkaloids, primarily including caffeine (130), theobromine (131), and theophylline (132)<sup>[25]</sup>. Among these, caffeine is the most abundant, accounting for approximately 1%–4% of the dry weight of tea leaves. Alkaloids are not only important for the flavor profile of the tea infusion but also play a critical role in its quality. The caffeine content directly affects the tea's quality: excessively high levels can make the tea overly bitter, while low levels may weaken its health benefits. Additionally, alkaloids serve as a vital nitrogen source for microorganisms during the pile fermentation process, playing a key role in the formation of the flavor and overall quality of dark tea.

In addition to the primary alkaloids, Tian et al.<sup>[18]</sup> discovered 7-methylxanthine (133), a precursor to caffeine, in dark tea. Furthermore, RPT has been found to contain 8-oxygenated caffeine and several pyrimidine alkaloids, including deoxythymidine (134),

thymine (135), and uracil (136)<sup>[28]</sup>. A novel alkaloid, N-(2-hydroxyphenyl)-2-pyrrolidinone (137), was also isolated from Qianliang tea<sup>[36]</sup>. Studies have shown that the inoculation of *Aspergillus sydowii* during the fermentation of RPT can increase the levels of purine alkaloids such as theobromine, 3-methylxanthine, and 1,7-dimethylxanthine, while simultaneously degrading caffeine<sup>[37]</sup> (Fig. 5).

## Terpenoids

Terpenoid compounds refer to olefinic compounds with molecular formulas that are integer multiples of isoprene. They are widely found in plants and are known for their significant biological activities. As a result, they are important sources for the development of natural functional ingredients, new drugs, and other bioactive compounds. Recently, a number of triterpenoid compounds have been extracted and characterized from dark tea. For example, Lin et al.<sup>[38]</sup> isolated three new triterpenoids from FZT:  $3\beta,6\alpha,13\beta$ -Trihydroxyolean-7-one (138),  $3\beta$ -Acetoxy- $6\alpha,13\beta$ -dihydroxyolean-7-one (139), and  $3\beta$ -O-(8-Hydroxyoctanoyl)-12-oleanene (140), as well as other compounds like Friedelin (141),  $\beta$ -Amyrone

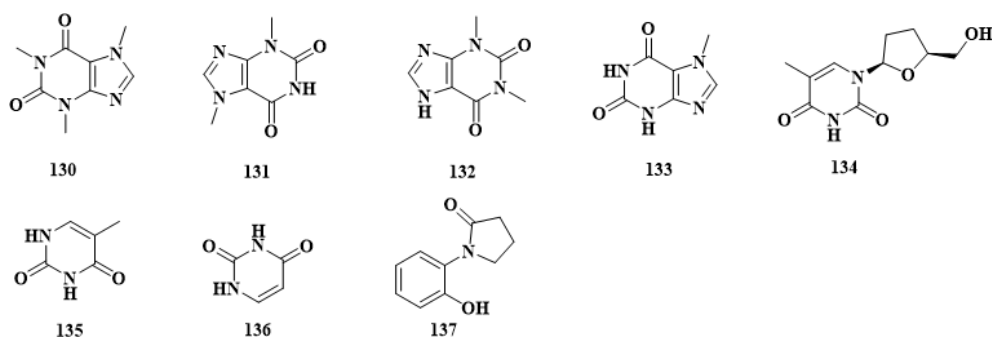


Fig. 5 Chemical structural formulas of alkaloids.

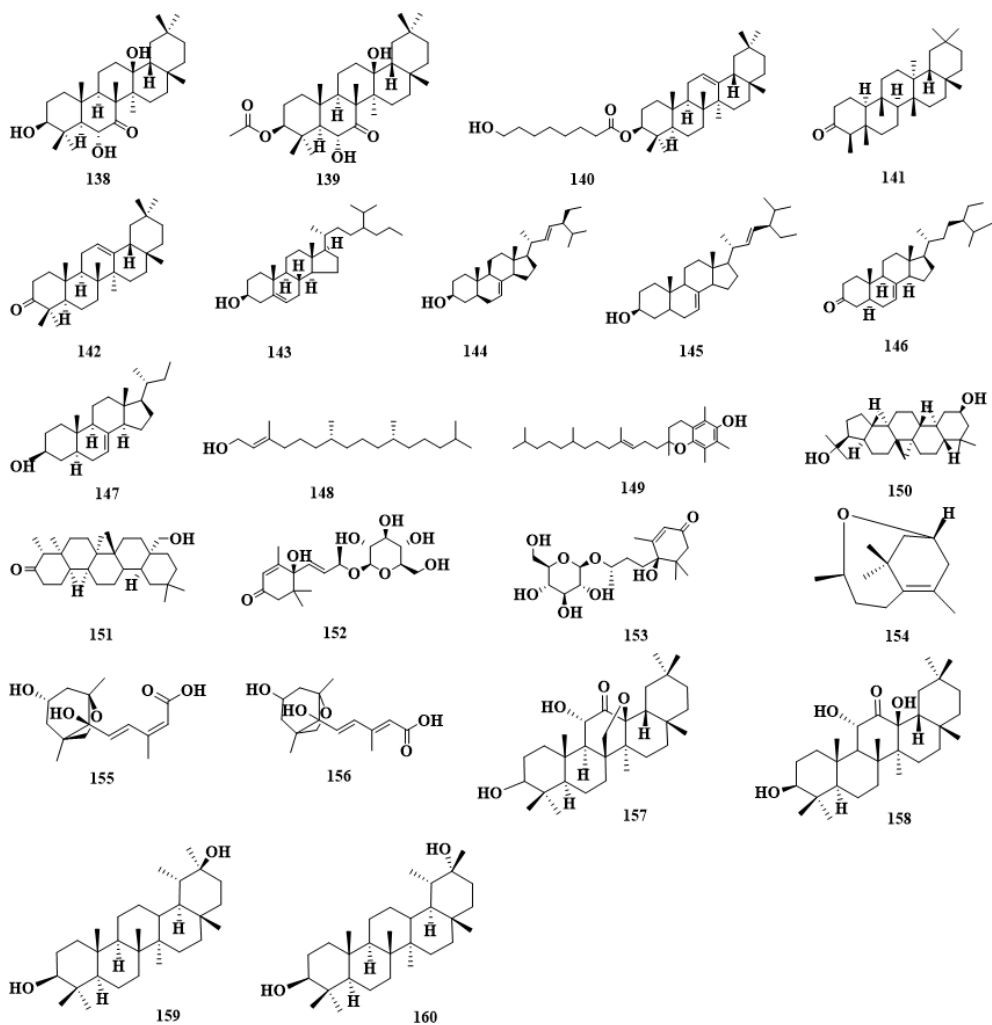


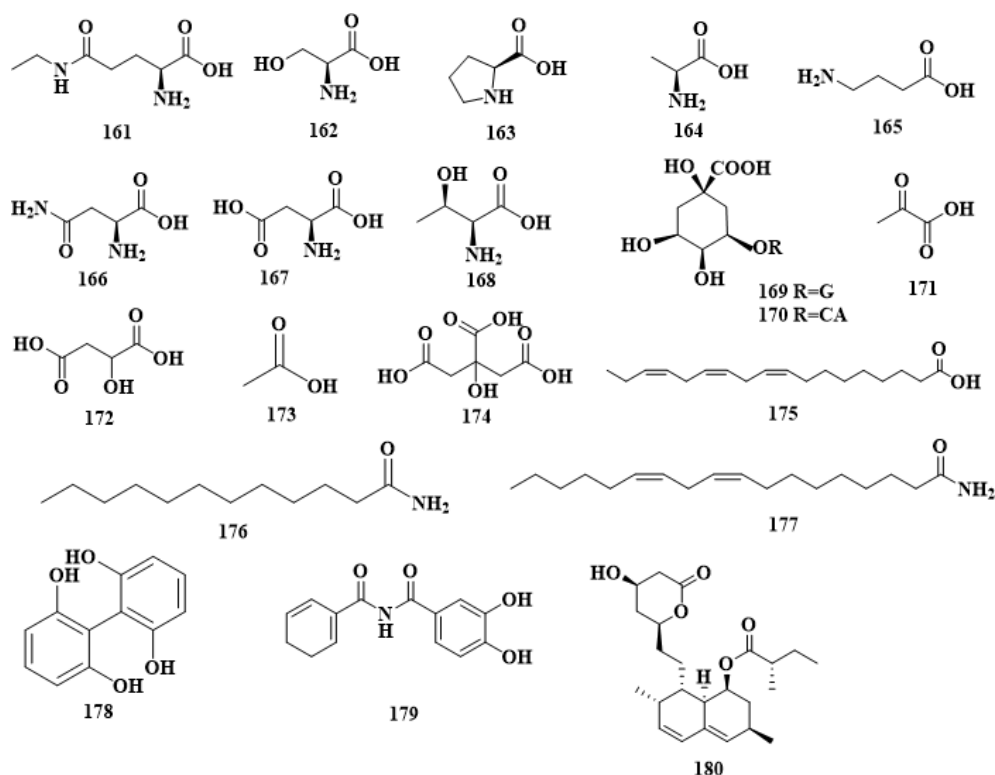
Fig. 6 Chemical structural formulas of terpenoids.

(142),  $\beta$ -Sitosterol (143),  $\alpha$ -spinasterone (144),  $\alpha$ -spinasterol (145), 22,23-dihydro- $\alpha$ -spinasterone (146), 22,23-dihydro- $\alpha$ -spinasterol (147), R-Phytol (148), and  $\alpha$ -tocopherol (149). Additionally, Zhu et al.<sup>[34]</sup> isolated two triterpenoid compounds from Jingwei FZT: 2-hydroxydiplopterol (150) and canophyllol (151). Furthermore, two new isoprenoid glycosides were identified from FZT: roseoside (152) and icariside B5 (153), as well as a novel isoprenoid compound, 3R,9R-oxido-5-megastigmenone (154)<sup>[26]</sup>, which is believed to be a precursor substance for aroma in fresh tea leaves. Two sesquiterpenoid compounds were also identified in FZT: dihydrophaseic acid (155) and 5-(3,8-dihydroxy-1,5-dimethyl-6-oxabicyclo[3.2.1]oct-8-yl)-3-methyl-2(E),4(E)-pentadienoic acid (156)<sup>[20]</sup>. Furthermore, two

new triterpenoid compounds were isolated from Japanese post-fermented tea (Awa-bancha): 13,26-Epoxy-3 $\beta$ ,11 $\alpha$ -dihydroxyolean-12-one (157), 3 $\beta$ ,11 $\alpha$ ,13 $\beta$ -Trihydroxyolean-12-one (158), as well as Taraxastane-3 $\beta$ ,20 $\beta$ -diol (159) and taraxastane-3 $\beta$ ,20 $\alpha$ -diol (160)<sup>[39]</sup> (Fig. 6).

## Amino acids

Tea leaves contain a variety of amino acids, with theanine being a distinctive amino acid found only in tea. Free amino acids play a crucial role in the aroma and taste of tea, being the primary source of its sweetness and umami flavor. The total free amino acid content



**Fig. 7** Chemical structural formulas of amino acids, organic acids, tea polysaccharides, and other compounds.

in RPT is 0.226%, with the predominant amino acids being theanine (161), serine (162), and proline (163), with the highest content of theanine<sup>[40]</sup>. Although FZT also contains a variety of amino acids, its overall content is relatively low. The main amino acids in FZT include alanine (164), gamma-aminobutyric acid (165), asparagine (166), theanine (161), aspartic acid (167), and threonine (168)<sup>[41]</sup> (Fig. 7).

## Organic acids

Tea leaves contain a rich array of organic acids, with over 25 types identified, including citric acid, oxalic acid, and malic acid. In RPT, Tian et al.<sup>[18]</sup> discovered two quinic acid derivatives, namely 3-O-polyacylquinic acid (169) and 3-O-caffeoylquinic acid (170). FZT also contains various organic acids, such as pyruvic acid (171), D-malic acid (172), acetic acid (173), and citric acid (174), with citric acid being the most abundant<sup>[42]</sup>. Additionally, several fatty acids have been found in FZT, including  $\alpha$ -linolenic acid (175), linoleamide (176), and dodecanamide (177)<sup>[43]</sup> (Fig. 7).

## Tea polysaccharides

Tea leaves contain a wide variety of carbohydrates, which can be classified into soluble sugars and insoluble sugars. Monosaccharides and disaccharides are the main components of soluble sugars and are the primary contributors to the sweet taste of tea, helping to alleviate the bitterness and astringency. Starch, cellulose, and pectin are the major polysaccharides in tea. FZT polysaccharides are a type of protein-bound acidic heteropolysaccharide, primarily composed of mannose, rhamnose, galacturonic acid, glucose, galactose, and arabinose<sup>[44]</sup>.

## Other compounds

RPT contains a variety of unique chemical compounds, including 2,2',6,6'-tetrahydroxybiphenyl (178)<sup>[16]</sup> and a novel amide

compound, N-(3,4-dihydroxybenzoyl)-3,4-dihydrobenzamide (179)<sup>[27]</sup>. Additionally, Hwang et al.<sup>[45]</sup> were the first to report the discovery of lovastatin (180) in RPT, which is the only statin found in RPT. Studies have shown that short-term fermentation of tea leaves using *Streptomyces bacillaris* or *Streptomyces cinereus* can increase the content of statin compounds (Fig. 7).

## Regulatory technologies

In recent years, with the optimization of key processing stages, both production efficiency and quality have seen significant improvements, especially in dark tea processing. This includes the regulation of harvesting, fixing, and rolling, as well as innovations in fermentation, microbial processes, and drying techniques. In tea processing, the innovation and application of artificial regulatory technologies, such as environmental control and microbiological techniques, play a crucial role in enhancing and stabilizing the quality of tea products. By fine-tuning these stages, the consistency and quality of tea products can be effectively improved.

## Harvesting, fixing, and rolling

The harvesting of dark tea is typically done by hand to ensure the integrity and consistency of the leaves. Unlike green and white teas, which are picked for their tender buds, the harvesting standard for dark tea mainly uses mature, full shoots as raw material. These shoots are typically composed of coarser leaves and longer stems, so it is normal to find tea stems in dark tea (with exceptions like PET). For example, LBT is harvested from mature leaves with one bud and two to three leaves, or sometimes one bud and four to five leaves<sup>[3]</sup>. This relatively mature harvesting standard means the leaves contain higher amounts of tea polyphenols, caffeine, and other substances, providing a rich material base for the subsequent fermentation process.

## Chemical composition and fermentation of dark tea

The picking standard also impacts the quality of the final dark tea. For high-quality dark teas, such as the Tianjian tea, the standard is typically one bud with one or two leaves just unfolding. These tender leaves are rich in tea polyphenols, amino acids, and other compounds, providing a fresh taste and intense aroma. For mid- to low-grade dark tea, the picking standard can be relaxed, with fresh leaves selected with one bud and two to three leaves. Although these leaves are less tender, careful processing control can still produce dark tea with a unique flavor. The season significantly impacts the quality of the harvest. Dark tea is typically picked in the spring and autumn. Spring-picked tea usually has a fresh, elegant fragrance with floral and fruity notes, while autumn-picked tea often has a stronger, richer aroma with hints of mature fruit. While mechanical harvesting is more efficient, it can damage the leaves, reduce the activity of tea polyphenol oxidase, and affect the fermentation process. Therefore, high-quality dark tea is still primarily hand-picked<sup>[46]</sup>.

The primary functions of fixing in dark tea production include facilitating the loss of water and softening of fresh leaves, inhibiting the oxidation of polyphenol oxidase, removing the grassy taste, and enhancing the aroma. Traditional fixing methods, such as steam fixing and sun drying, are prone to losing aroma or are restricted by environmental conditions. In contrast, modern hot air fixing technology achieves uniform heating through a staged temperature rise (60 °C → 80 °C → 100 °C), which significantly improves the quality of dark tea. This technology can increase the content of aromatic compounds in dark tea by over 30%, improve the retention rate of catechins by 15%, and promote appropriate cell damage, creating favorable conditions for subsequent fermentation<sup>[47]</sup>. Moreover, the duration of the fixing process directly impacts the quality of the tea. An appropriate fixing time ensures the complete deactivation of enzymes while avoiding excessive heating that could degrade the tea's quality. By precisely controlling the fixing time, the optimal tea quality can be achieved, thereby optimizing the overall processing effect.

Fixing can be divided into manual and mechanical methods, with mechanical fixing being the more commonly used approach today. The fixing equipment primarily includes drum-type, steam-type, microwave-type, far-infrared-type, and hot air-type machines. With the rapid development of the tea industry, single traditional fixing machines are increasingly unable to meet the basic processing needs of tea leaves. Modern research has focused on technologies such as hot-air recirculation drum fixing machines and steam-hot-air

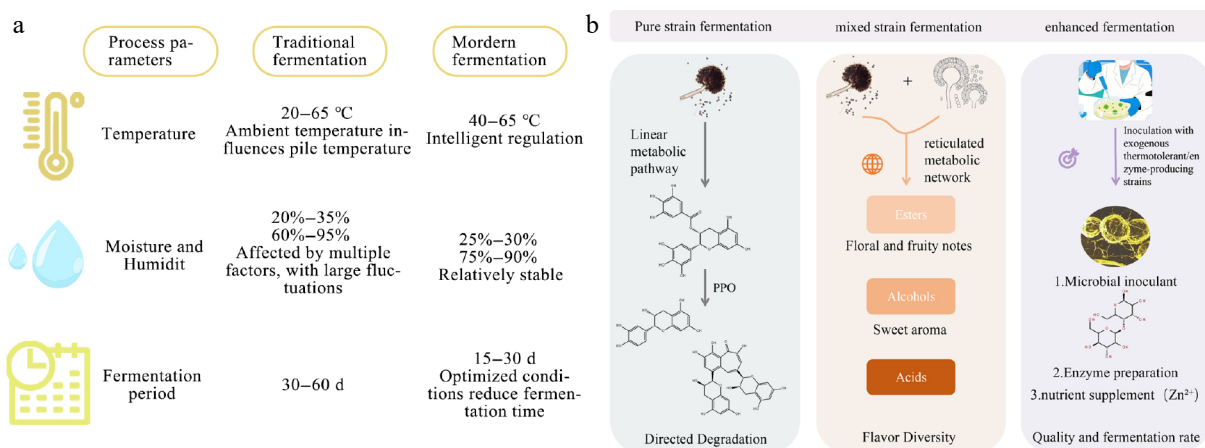
coupling drum fixing machines to improve efficiency and the stability of quality. Experiments have shown that using a superheated steam-high-temperature hot air-drum coupling process (with a drum speed of 28 r/min) achieves the highest fixing efficiency, with tea polyphenol and amino acid contents reaching 17.48% and 3.78%, respectively<sup>[48]</sup>.

The raw material of dark tea is relatively coarse and mature, and rolling is a step in the processing. The goal is to gently break the cells to release tea juice and shape the leaves into twisted strips for fermentation<sup>[49]</sup>. The intensity of the kneading process should be adjusted based on the tenderness of the fresh leaves and the quality requirements of the dark tea. For tender leaves, the kneading intensity should be light, typically using a light press and short kneading method, with kneading time controlled between 10 and 15 min to avoid excessive cell breakage and an increase in leaf fragments. For older fresh leaves, the kneading intensity can be slightly increased, and the kneading time extended to 20 to 30 min, allowing the cells to break fully and release juice, which helps with the fermentation during the subsequent piling process.

During kneading, it is essential to regularly check the degree of kneading. In the initial rolling, the machine speed is set around 40 r/min, and the process lasts about 15 min to achieve 20% cell damage, ensuring that the tea juice is evenly distributed on the leaf surface. Re-rolling is done after pile fermentation with additional pressing for 6 to 8 min, which tightens the leaves and improves the release of internal compounds. Rolling causes moderate damage to the leaves, releasing amino acids and soluble sugars, which further transform during fermentation to create the fresh and rich flavor of dark tea. Amino acids enhance the tea's freshness, while soluble sugars contribute to its sweetness and smoothness. Therefore, precise control of the rolling process is crucial for the flavor and quality of dark tea.

## Fermentation process optimization

Pile fermentation is the most critical step during the dark tea processing. The quality of dark tea during the pile fermentation process is mainly influenced by factors such as moisture, temperature, fermentation time, and microorganisms. Suitable levels of moisture, temperature, and fermentation time promote microbial growth, which, through microbial metabolism and enzymatic actions, leads to the transformation of chemical components in the tea (Fig. 8a).



**Fig. 8** Fermentation environmental factors and microbial regulation in dark tea. (a) Environmental factors in dark tea fermentation. (b) Microbial regulation in dark tea: pure culture, mixed fermentation, and fortified fermentation.

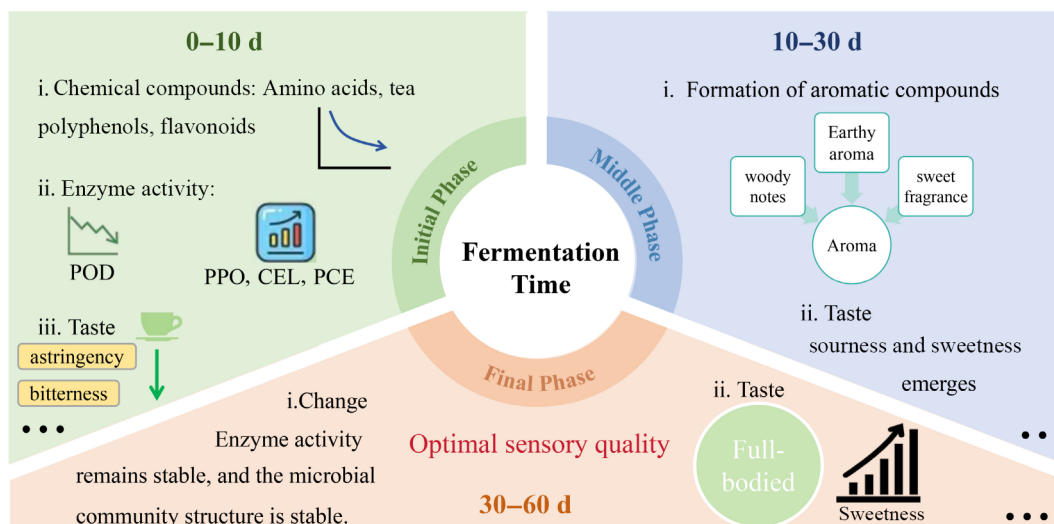


Fig. 9 Effects of fermentation time.

### Fermentation time

Fermentation time is one of the key factors in the entire pile fermentation process, playing a decisive role in its sensory quality and chemical composition. Insufficient fermentation results in cloudy tea liquor, weak aroma, and a bitter taste, while over-fermentation may lead to off-flavors such as sourness due to excessive microbial growth. The fermentation process can be divided into three stages: early (0–10 d), middle (10–30 d), and late (30–60 d)<sup>[50]</sup> (Fig. 9). In the early stage, fermentation time can be appropriately shortened to promote the establishment of microbial communities and initial metabolic activities; in the middle stage, it can be extended to facilitate the accumulation of metabolic products; and in the late stage, when the flavor and quality are maturing, shortening the fermentation time can help prevent over-fermentation. By controlling fermentation time in different stages, microbial succession and the accumulation of metabolic products can be better regulated.

Studies show that fermentation time directly affects metabolic transformations in tea, such as polyphenol degradation, organic acid accumulation, and the formation of microbial metabolites like coenzyme Q10<sup>[51]</sup>. For example, extending fermentation time in RPT enhances organic acid diversity, giving the tea a smoother taste<sup>[52]</sup>, while QZT enters a critical transformation phase around 17 d, at which point its aroma and flavor compounds stabilize<sup>[53]</sup>. These compounds have been shown to interact with other volatile components, creating more complex and appealing aromatic profiles.

Fermentation time also regulates microbial community succession. A moderate extension of fermentation promotes the growth of beneficial fungi like *Aspergillus* and *Eurotium*<sup>[54]</sup>. However, the microbial succession is dynamic and can influence the final chemical composition, including the generation of specific volatile compounds that contribute to the tea's characteristic aroma. Over-fermentation can lead to an imbalance in microbial populations, potentially increasing the number of spoilage organisms, which not only affects flavor but also compromises the safety of the tea. For example, extended fermentation in RPT can enhance the synthesis of methoxyphenyl compounds, which significantly enhance its aroma<sup>[55]</sup>. The length of fermentation (0, 3, 6, 9, or 12 h) significantly influences the microbial community and its biochemical composition<sup>[56]</sup>.

During fermentation, regular sensory evaluations are essential to ensure that the resulting dark tea has a desirable flavor and quality.

Sensory assessment helps adjust the fermentation time in real-time to achieve optimal quality. As fermentation progresses, the bitterness and astringency of the tea gradually decreases, while sweetness and smoothness increases<sup>[56]</sup>. The fermentation process of dark tea continues during storage, and over time, the degree of fermentation increases, leading to an improvement in flavor. In the case of PET, *Aspergillus niger* can convert tannins into gallic acid, resulting in a milder taste. Additionally, *A. niger* produces various hydrolytic enzymes that influence the flavor and quality of PET<sup>[57]</sup>. Therefore, an appropriate fermentation period provides favorable conditions for the tea's later 'aging', enhancing both its taste and aroma.

In practical production, fermentation time is typically controlled between 15 and 60 d, depending on temperature, humidity, and raw material characteristics. High temperature and humidity (25–30 °C, 80%–85% relative humidity) can significantly shorten the fermentation cycle<sup>[58]</sup>, while larger-leaf varieties require a longer fermentation time<sup>[50]</sup>. Under optimal temperature and humidity conditions, 60 d of fermentation are considered the ideal fermentation time for dark tea<sup>[59]</sup>. Therefore, optimizing fermentation time and process parameters is crucial, not only to improve the flavor of dark tea but also to enhance its health benefits, such as antioxidant capacity, fat reduction, weight loss, and anti-aging properties.

### Moisture and humidity

Moisture plays a crucial and multifaceted role in the pile fermentation process of dark tea. It serves not only as a physical medium but also as a direct and essential participant in the complex chemical transformations occurring within the tea leaves<sup>[60]</sup>. Moisture influences a range of critical processes, including the rate of enzymatic and biochemical reactions, the modulation of microbial growth and metabolism, and the alteration of microbial community composition. These factors collectively shape both the progression and the final quality of the fermentation. During the early stages of fermentation, an adequate moisture content is essential for the activation of enzymes such as oxidases and hydrolases. This activation accelerates the oxidation of tea polyphenols, a key process in the formation of the characteristic color, flavor, and aroma profiles that define high-quality dark tea. As fermentation progresses into its later stages, it is necessary to reduce the moisture content to prevent over-fermentation. Excessive moisture may lead to undesirable changes in the flavor profile and overall quality of the tea. Throughout the fermentation process, key physicochemical

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parameters such as pH, moisture content, liquor color, and levels of water-soluble extracts undergo dynamic changes. These changes are not incidental; rather, they provide valuable insights that can serve as markers for monitoring fermentation progress<sup>[61]</sup>, and for optimizing control parameters to ensure the desired outcome.

As fermentation continues, moisture content naturally decreases due to evaporation and microbial activity within the tea pile. Consequently, to maintain optimal microbial activity and preserve the biochemical balance during fermentation, replenishing moisture through spraying or humidification becomes essential. Research by Luo et al.<sup>[61]</sup> showed that during the fermentation of QZT, the water-extractable content of the tea decreased rapidly in the early stages, followed by a slower, more gradual decline as fermentation advanced. Similarly, Wen et al.<sup>[62]</sup> reported that by maintaining a relative humidity of 20%–25% through regular water spraying, they successfully stabilized the tea's moisture content at approximately 26%, which positively influenced both the flavor and aroma of the tea. However, excessive moisture (typically greater than 30%) can create anaerobic conditions that inhibit enzymatic activity and disrupt microbial metabolism<sup>[63]</sup>. Conversely, insufficient moisture content, typically below 20%, can result in fermentation stagnation, leading to suboptimal tea quality.

Turning the tea piles is also an essential practice for ensuring proper moisture distribution and regulating the thermal environment within the pile. Typically, three to four turnings are performed during fermentation to prevent localized over-drying or over-wetting, ensuring more uniform fermentation throughout<sup>[64]</sup>. The initial moisture content of the raw materials also plays a crucial role in determining the fermentation outcomes. Chen et al.<sup>[65]</sup> found that wet pile fermentation typically begins with fresh tea leaves containing approximately 60% moisture, whereas dry pile fermentation uses tea leaves with less than 30% moisture, achieved through sun-drying or baking. Wet pile fermented teas tend to have higher levels of TBs, a characteristic pigment, while dry pile fermentation favors the accumulation of certain lipids and organic acids, which contribute to different flavor and chemical profiles.

Humidity, as an external environmental factor, is equally critical to the fermentation process. Proper humidity levels promote microbial growth, enhance enzymatic activity, and accelerate the transformation of polyphenols and catechins, all of which are essential for developing the characteristic attributes of dark tea. However, excessive humidity can promote mold contamination, while insufficient humidity can suppress microbial activity and hinder fermentation. Liu et al.<sup>[66]</sup> demonstrated that a relative humidity range of 65%–75% significantly enhances microbial diversity, which in turn improves the chemical composition of the tea, ultimately resulting in higher quality tea with enhanced flavor and aroma characteristics. Li et al.<sup>[67]</sup> reported that the optimal moisture content for pile fermentation of dark tea is approximately 38 g per 100 g of wet tea leaves, maintained under a relative humidity of 85%. Under these conditions, the levels of tea polyphenols (particularly ester catechins), amino acids, and soluble sugars decrease, while characteristic pigments such as TBs and TRs increase. These pigments are key to the tea's color and flavor profile.

In conclusion, achieving precise control over both the internal moisture content of the tea and the ambient humidity in the fermentation environment is critical for producing high-quality, standardized dark tea. Furthermore, integrating real-time monitoring systems to track moisture levels, temperature fluctuations, and microbial profiles throughout the fermentation process could significantly enhance quality control and overall efficiency in industrial-scale dark tea production. Such technological

advancements would refine the fermentation process, ensuring that the tea produced consistently meets high standards in terms of flavor and aroma.

### Temperature

Temperature plays a critical role in the fermentation process of dark tea, influencing microbial activity, enzymatic reactions, and the biochemical transformation of tea compounds. Precise temperature control is essential to maintain optimal fermentation conditions. Temperatures that are too low can slow down microbial and chemical processes, while excessively high temperatures may inhibit enzyme activity, causing thermal degradation and undesirable 'burnt' flavors. For example, high-temperature fermentation can significantly alter components such as amino acids<sup>[40]</sup>. A slight increase in temperature can enhance the fermentation activity of *Eurotium cristatum* in FZT, improving its antioxidant capacity and increasing gallic acid content<sup>[68]</sup>. Additionally, drying temperature impacts the quality of dark tea. Research indicates that the best tea quality is achieved when dried at 60 °C<sup>[69]</sup>. Therefore, maintaining an appropriate fermentation temperature is crucial for the development of high-quality tea.

Fermentation temperature has a profound effect on the color development of dark tea. At optimal temperatures, the enzymatic oxidation and polymerization of tea polyphenols lead to the formation of pigments such as TFs, TRs, and TBs, which contribute to the characteristic reddish-brown or dark brown hue of high-quality dark tea. Low temperatures can result in incomplete pigment formation, leading to a lighter color, while excessively high temperatures can cause over-darkening, diminishing the tea's visual appeal. For specialty teas like Anhua dark tea, the control of fermentation temperature also affects the color and distribution of the 'golden flowers' (*E. cristatum*) on the surface. An appropriate temperature promotes the growth and reproduction of the 'golden flowers', resulting in a desirable color and distribution, which in turn enhances the visual quality of the tea.

Fermentation temperature significantly influences the flavor and aroma profile of dark tea. The regulated oxidation and polymerization of polyphenolic compounds facilitate the development of a smooth, mellow, and slightly sweet taste in the tea liquor. Temperature also modulates the concentrations of amino acids and soluble sugars, which further contribute to mouthfeel and overall flavor balance. Moreover, microbial and enzymatic activities convert aroma precursors into volatile compounds (such as alcohols, aldehydes, and esters), which collectively define the tea's aromatic profile. Proper control of fermentation temperature facilitates the development of preferred aroma characteristics, including mature, woody, and sweet notes.

During the fermentation process, precise regulation of both ambient and internal pile temperatures is essential. Empirical studies have identified the optimal environmental temperature range for dark tea fermentation to be 28–35 °C, whereas the internal pile temperature should be maintained between 35 and 70 °C<sup>[64]</sup>. Elevated pile temperatures enhance the activity of beneficial microorganisms while concurrently inhibiting the proliferation of undesirable microbes. As fermentation proceeds, microbial metabolism generates heat, which further elevates the internal pile temperature and influences subsequent fermentation dynamics. For instance, microorganisms such as *Aspergillus* and *Bacillus* decompose organic matter within tea leaves, releasing heat that contributes to an increase in pile temperature<sup>[50]</sup>. Different types of dark tea exhibit distinct optimal fermentation temperature requirements. For example, the ideal pile temperature for FZT ranges from 40–50 °C,

while QZT requires a higher range of 50–65 °C. The fermentation temperature for LBT can reach up to 55 °C<sup>[70]</sup>. Additionally, specific fermentation methods demand unique temperature conditions: for instance, LBT pile fermentation should be maintained around 50 °C, whereas steam-assisted fermentation typically necessitates a higher temperature of approximately 80 °C<sup>[3]</sup>.

In conclusion, fermentation time, moisture, and temperature are critical factors in determining the color, aroma, taste, and overall quality of dark tea. These parameters interact in complex ways, influencing microbial activity, enzymatic processes, and the biochemical transformations that shape the sensory attributes of the tea. While current research often isolates these factors for study, it is essential to recognize their integrated effects during fermentation. Therefore, establishing multi-parameter regulation frameworks is crucial to improve fermentation precision and standardization. The use of real-time monitoring technologies, such as high-throughput microbial sequencing, near-infrared spectroscopy, and thermal imaging, can provide dynamic insights into the fermentation process, enabling adaptive control over temperature, humidity, and fermentation time.

### Application of new technologies

In addition to new fermentation methods, some improved fermentation processes can significantly enhance the quality of dark tea. Building on traditional fermentation techniques, new technologies such as cold-water fermentation<sup>[71]</sup> and barrel fermentation<sup>[72]</sup> have been widely adopted in production. With advancements in technology, the fermentation process of dark tea has also incorporated methods such as electronic noses, spectroscopy, the Internet of Things (IoT), and machine learning, allowing for the visualization and intelligent control of the fermentation process. For example, Hu et al.<sup>[73]</sup> developed a fermentation process visualization technology that combines an electronic nose and spectroscopy to effectively detect the relationship between fermentation degree and quality characteristics. Furthermore, Hong et al.<sup>[74]</sup> developed an intelligent fermentation control system by integrating IoT and machine learning technologies, enabling real-time monitoring and automatic adjustment of parameters such as temperature, humidity, and oxygen concentration in the fermentation environment.

### Microbial community

During the dark tea processing, microorganisms catalyze a series of reactions, including oxidation, degradation, condensation, and structural modifications, leading to significant changes in the types and concentrations of compounds. For instance, most fungal and bacterial species in dark tea are associated with amino acids, catechins, TBs, and volatile acids in the tea leaves<sup>[75]</sup>. During fermentation, the polyphenol content significantly decreases<sup>[53]</sup>, primarily due to the oxidation of polyphenols and microbial metabolism. Furthermore, the A-ring or B-ring flavan-3-ol derivatives produced during microbial fermentation are key contributors to the reduction of catechins. Under microbial influence, the contents of certain flavonoids, phenolic acids, purine alkaloids, and amino acids in dark tea also change<sup>[7]</sup>. For example, research by Li et al.<sup>[76]</sup> found that during microbial fermentation, the levels of flavonoids and flavonols in dark tea decrease, while the concentrations of TBs, gallic acid, caffeine, and TRs increase with fermentation time. Thus, the variation and dynamic shifts in microbial communities are essential factors in shaping the quality of tea.

The diversity of microorganisms and the interaction between bacterial and fungal communities with dark tea samples and their pile fermentation environment are highly complex. Different fungi

and bacteria dominate in various dark tea pile fermentation processes.

In RPT, the dominant microbial genera are *Aspergillus*, *Bacillus*, and Enterobacteriaceae<sup>[77]</sup>, with *A. niger* being considered the main species. During the pile fermentation process, the abundance of different microbial species changes according to the fermentation stage: early on, *A. niger* rapidly increases in number, then gradually decreases in the later stages, while *Saccharomyces cerevisiae* numbers start low but increase slowly as fermentation progresses. Microbial fermentation is a key factor in the formation of RPT's quality. *A. niger* plays a leading role in the fermentation process, secreting enzymes like polyphenol oxidases that facilitate the conversion of tea compounds. Studies show that inoculating specific microorganisms, such as *Aspergillus* spp., during spontaneous pile fermentation and artificial solid-state fermentation can significantly increase the production of TBs<sup>[78]</sup>. Additionally, Wang et al.<sup>[79]</sup> first identified *A. marvanovae* and *Candida mogii* during the fermentation process of RPT.

The main microorganisms in FZT include fungi such as *Scopulariopsis*, *Debaryomyces*, and *Aspergillus*, as well as bacteria from genera like *Klebsiella*, *Lactococcus*, and *Bacillus*<sup>[80]</sup>. The dynamic changes in microbial communities during FZT fermentation are significant. During the pile fermentation stage, the primary microorganisms are *S. cerevisiae*, *A. niger*, *Penicillium*, and *Rhizopus*. As the tea enters the special 'flowering' fermentation process, large amounts of *E. cristatum* (commonly known as 'golden flowers') grow on the surface and inside the tea. This is the main dominant species during the fermentation process, accounting for over 95% of the microbial population and remaining throughout the 'flowering' period<sup>[81]</sup>. In addition to *E. cristatum*, other microorganisms are also present in FZT, including non-*Aspergillus* species such as *A. niger*, *Mucor*, *Penicillium*, *P. oxalicum*, and *P. brevicompactum*, as well as a diverse bacterial community. Moreover, inoculating with dominant species such as *E. cristatum* and *Debaryomyces* can significantly accelerate the fermentation process of dark tea and improve its quality<sup>[82]</sup>. These microorganisms may be involved in the synthesis of key flavor or aroma compounds in FZT, thus influencing its flavor and quality.

Research shows that, in the early fermentation stages of LBT, the dominant microorganisms are *Sphingomonas*, *Methylobacterium*, *Chryseobacterium*, *Aspergillus*, *Cladosporium*, *Penicillium*, and *Saccharomyces*. In the later stages of fermentation, the dominant microorganisms shift to *Staphylococcus*, *Brachy bacterium*, *Kocuria*, *Saccharomyces*, and *Aspergillus*. These microbial communities are significantly related to the transformation of tea polysaccharides, catechins, flavonoids, and caffeine in LBT<sup>[83]</sup>.

Various dominant microbial species are also present in other brick teas, such as Kangzhuan brick tea and QZT. In the fermentation of QZT, the primary fungal species include *S. cerevisiae* (such as *Cyberlindnera*), *Aspergillus*, while the dominant bacterial genera include *Klebsiella*, *Paenibacillus*, *Cohnella*, and *Pantoea*<sup>[84]</sup>. Additionally, the dominant microbial genera in Kangzhuan brick tea are *Staphylococcus* and *Bacillus*. Studies have shown that *Bacillus subtilis* is among the predominant bacterial strains involved in TT fermentation<sup>[85]</sup>.

### Microbial regulation

Traditional fermentation technologies have certain inherent limitations. For example, the traditional fermentation process involves various microorganisms from the environment, which can trigger complex chemical transformations. Due to the inability to precisely control this process, the quality of dark tea often fluctuates, and there is a risk of microbial contamination<sup>[86]</sup>. To overcome the

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shortcomings of traditional fermentation, researchers have developed several new fermentation techniques, such as pure strain fermentation, mixed strain fermentation, and enhanced fermentation. These methods involve selecting and using specific microbial communities, such as *S. cerevisiae*, *Klebsiella*, *E. cristatum*, etc., to replace the uncertainty of microorganisms from the natural environment in traditional fermentation, thereby improving the quality and health benefits of dark tea.

Pure strain fermentation uses a single strain to carry out the fermentation, effectively avoiding the contamination risks posed by complex microbial communities in traditional fermentation. This method also allows for more precise control over the fermentation conditions. For example, using *A. niger* for pure strain fermentation can significantly increase the content of TBs, thereby enhancing the color, flavor, antioxidant capacity, and lipid-lowering effects of dark tea<sup>[86]</sup>. Further research has shown that the same strain, under different fermentation conditions, can lead to significant differences in the flavor profile of the tea. For instance, RPT fermented with solid *Monascus purpureus* has a stronger floral aroma, while RPT fermented in liquid form develops a more pronounced aged flavor<sup>[87]</sup>.

Mixed-strain fermentation refers to the simultaneous use of two or more strains for fermentation. This method takes advantage of the synergistic effects between different strains, leading to the production of more complex metabolites that enhance the flavor and aroma of dark tea. For example, Chen et al.<sup>[88]</sup> used a mixed fermentation of *A. niger* and *A. cristatus*, resulting in dark tea with a richer array of volatile compounds, such as  $\alpha$ -terpineol and phenylethanol. Additionally, Xu et al.<sup>[89]</sup> found that a mixed fermentation of *A. fumigatus* and *Bacillus subtilis* significantly increased the content of TBs, improving both the flavor and color of dark tea.

Enhanced fermentation involves the addition of exogenous functional strains to speed up the fermentation process, reduce fermentation time, and improve production efficiency. For instance, using *Monascus purpureus* in enhanced fermentation can significantly increase the lovastatin content in dark tea, thereby enhancing its cholesterol-lowering effects<sup>[90]</sup>. Yu et al.<sup>[91]</sup> demonstrated that enhancing the fermentation of RPT with *Penicillium simplicissimum* resulted in a significant reduction in caffeine content, improving both the flavor and health benefits. Enhancing fermentation with *Bacillus licheniformis* to improve the antioxidant activity and intestinal barrier protection of TT<sup>[85]</sup>. Furthermore, during the solid-state fermentation phase, *A. niger* can convert catechins into TBs, while in the liquid-phase fermentation, the content of TBs and their antioxidant activity is further enhanced<sup>[92]</sup>.

As research on the key microorganisms and functional activities in dark tea deepens, the potential of exogenous microbial inoculation techniques in dark tea production is becoming increasingly evident. Notably, the practice of inoculating the 'golden flower' fungus has emerged as a highly valuable technique. Studies have shown that this technique not only effectively improves the sensory quality of dark tea but also significantly shortens the fermentation cycle<sup>[60]</sup>, providing dual benefits for the efficiency and quality of dark tea production (Fig. 8b).

## Drying

Drying is the final step in the initial processing of dark tea, and it plays a crucial role in developing its distinct characteristics, such as its dark, glossy appearance and unique smoky aroma. Traditional drying methods, such as sun-drying and air-drying, vary by region. However, these methods are highly dependent on weather conditions and can be difficult to control in terms of hygiene. With the

advancement of technology, modern methods such as microwave drying and far-infrared drying have been introduced to improve the tea drying process.

Microwave-assisted drying has been shown to accelerate chemical reactions among the compounds in tea, altering the physical properties of volatile components and enhancing the aroma, making it fresher and longer-lasting<sup>[69]</sup>. This is especially important as the aroma of dark tea evolves through various stages of processing, including fermentation, drying, and post-storage. Drying, in particular, is a critical step that influences both the aroma and taste of the final product. Studies suggest that methoxyphenols, characteristic aromatic compounds, may be linked to methylation reactions involving catechins during fermentation, drying, and storage<sup>[93]</sup>.

Different drying methods have a significant impact on the quality of tea. Orange peel dark tea, when dried using hot air, outperforms sun-dried tea in terms of sensory qualities, chemical composition, and bioactivity. Specifically, low-temperature drying (40–50 °C) effectively reduces bitterness and astringency in orange peel dark tea, while increasing the levels of polyphenols, flavonoids, hesperidin, and free amino acids, which significantly enhance the overall quality of the tea<sup>[94]</sup>.

The temperature during drying is a critical factor in ensuring the tea's quality. The tea leaves are typically left to rest for 1–2 d to balance the moisture inside and outside the brick, before gradually increasing the temperature for drying. The temperature is then gradually increased. For QZT, the drying process follows a temperature gradient: starting from 35–40 °C, rising to 40–55 °C, and eventually reaching 55–65 °C. The final moisture content of the tea should be below 12%<sup>[95]</sup>.

Aging is another essential process that helps develop the mellow, aged aroma of dark tea. The aging process involves environmental factors that trigger oxidation and polymerization reactions in the tea's compounds, thereby forming its unique flavor and quality characteristics. The longer the aging period, the better the flavor and quality of the tea, particularly for FZT<sup>[43]</sup>. For QZT, when dried with hot air, it develops a distinct aged aroma and shows excellent overall quality, with an increase in free amino acids. Furthermore, aging helps to reduce bitterness and astringency, while enhancing the tea's smoothness, sweetness, and richness<sup>[95]</sup>. Aging involves storing the dried tea leaves in a cool, moist environment for at least six months, with natural aging typically requiring three to five years to develop the tea's unique aged flavor<sup>[96]</sup>.

## Conclusions

This review summarizes the unique chemical components and control technologies of dark tea. The distinctive flavor and sensory qualities of dark tea arise from the synergistic interaction of its unique chemical compounds. Currently, research on dark tea's quality focuses on two main areas: identifying its chemical foundation through component analysis, and understanding how processing conditions like temperature, humidity, fermentation time, and microbial communities impact quality. These insights help optimize production processes and product development. Control technologies in dark tea processing, such as harvesting, fixing, rolling, fermentation, and drying, play a crucial role in shaping its quality. Proper management of these stages, especially fermentation conditions, ensures the development of dark tea's unique flavor and aroma. As interest in dark tea continues to grow, there has been an increasing volume of research. However, several aspects should be considered for future studies.

At present, the exact reactions during the fermentation process remain unclear. Moreover, several new compounds have been identified in dark tea, and further in-depth studies on their bioactivity and sensory evaluation are necessary. The structural and functional relationships of some key components, such as TBs, remain unresolved. While TBs have been shown to be crucial for color and taste formation, their molecular structure and physicochemical properties have not been fully elucidated<sup>[12]</sup>. Therefore, future research should further integrate modern analytical techniques (mass spectrometry) to deepen the study of dark tea's chemical components, providing a theoretical basis for targeted quality control.

Additionally, current studies are mainly focused on RPT, FZT, and LBT, with limited research on other types of dark tea, such as Hei zhuan brick tea, Qian liang tea, Tian jian tea, QZT, and Kangzhuan brick tea. Future studies should expand to include these lesser-explored types, particularly regarding their chemical profiles, fermentation microorganisms, health benefits, and quality development mechanisms. In recent years, certain single or mixed microbial strains isolated from dark teas have been employed in fermentation processes, resulting in products of comparable or superior quality to commercially available dark teas. Traditional fermentation methods, which rely on environmental microorganisms, often introduce variability. However, by selecting specific microbial communities—such as *S. cerevisiae*, *Klebsiella*, and *Clostridium*—and utilizing techniques like pure strain fermentation, mixed strain fermentation, and enhanced fermentation, the quality and health-promoting properties of dark teas can be significantly improved.

With advancements in mechanical engineering, artificial intelligence (AI), the Internet of Things (IoT), and blockchain, tea processing equipment is becoming more continuous, automated, and intelligent, greatly improving efficiency and quality control. In fermentation, technologies such as synthetic microbial communities and targeted enzymes help stabilize product quality. Ongoing improvements in dark tea fermentation equipment have enhanced process uniformity and significantly reduced manual labor intensity, showcasing the practical value of intelligent systems in production. Smart monitoring tools—including AI models and IoT sensors—also address challenges like strain degradation and high energy use, with such systems currently accounting for 15%–20% of production costs. Additionally, blockchain-based traceability and data security are fostering deeper integration between traditional processing and digital systems, accelerating the industry's shift toward smarter and higher-quality development.

Future research should focus on developing low-energy intelligent processing systems, constructing collaborative innovation mechanisms between industry, academia, and research, and achieving comprehensive digital empowerment. In-depth exploration of intelligent and automated technologies will be key to driving quality improvement and efficiency enhancement in the dark tea industry through technological innovation.

## Author contributions

The authors confirm contributions to the paper as follows: writing—original draft preparation: Chen H; writing—review and editing: Chen H, Li Y, Liang Y; investigation: Chen H, Chen Y, Ma M; supervision: Liu Z, Wang K, Huang J, Zhu M; conceptualization: Zhu M, Wang K; project administration: Zhu M; validation: Zhu M. All authors reviewed the results and approved the final version of the manuscript.

## Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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