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ORIGINAL ARTICLE

Chemical Composition and Sensory Evaluation of Fermented Tea with Medicinal Mushrooms

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Abstract In commercial tea production, plenty of tea leaf waste is generated, which may not only exert pollution risk to environment, but also a huge waste of bioactive ingredients in tea. In this study, the 4th to 7th leaves of tea bush were collected and used as substrate for mycelial culture of two renown medicinal mushrooms *Grifola frondosa* and Tianzhi (new variants of *Ganoderma lucidum*) to obtain a new type of solid-state fermented tea. Result showed that the polysaccharides of *Grifola frondosa* and Tianzhi fermented tea were 1.52 and 4.14 %, tea polyphenols were 1.51 and 1.85 %, the free amino acids were 1.52 and 0.94 %, caffeine were 1.16 and 1.70 %, polyphenols/amid acids ratio were 1.0 and 1.98, water extractions were 35.53 and 32.86 %, protein contents were 17.63 and 6.13 mg/g, respectively. The volatile components were mainly composed of alcohols, esters, aldehydes and ketones. The contents of major flavor compositions of fermented tea had changed and their relation tended to be harmonious, and the variety of amino acids significantly increased. Therefore, the sensory flavor and therapeutic qualities of fermented tea were significantly improved.

Keywords Fermentation tea · Medicinal mushroom · Sensory evaluation · Polyphenols/amino acids ratio

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Introduction

Grifola frondosa, commonly known as maitake, is one of the most popular edible mushrooms. It is a nutritious food source and is reported to contain bioactive metabolites that exhibit many medicinal effects including anti-tumor, hypocholesterolemic, antioxidant and anti-diabetic activities [1–3].

Ganoderma lucidum, commonly called Mesona chinensis, is a basidiomycetous mushroom (*Polyporaceae*), which has been used for thousands of years as a traditional food and medicine, especially in China and other eastern Asian countries [4]. The fruiting bodies or spores of *G. lucidum* were reportedly effective in modulating the immune system, inhibiting tumors, and treating hyperglycemia and inflammatory diseases. *G. lucidum* has been widely used as a key ingredient in many traditional Chinese medicinal prescriptions or nutritional supplements [5–7].

Tea is a traditional beverage of China with numerous well-documented health benefits [8]. It is composed of polyphenols, alkaloids (caffeine, theophylline, and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds (chemicals that readily produce vapors and contribute to the odor of tea), fluoride, aluminum, minerals, and trace elements [9–12]. Especially among these compounds, tea polyphenols exhibit many properties such as free radical scavenging, anti-oxidation, anti-cancer, blood fat and cholesterol reduction, and atherosclerosis and antibacterial resistance [13].

In commercial tea production, only the first to third youngest leaves of the tea bush are usually used, thus about 30 % of tea waste leaves (including trimming leaves, tea dust and mature leaves) was produced annually, most of which is burned, dumped into landfills or used as compost [14]. This problem is remarkably observed in the largest tea-growing provinces of Yunnan, Fujian and Zhejiang in China. The first two of these waste management practices seem to have a seriously negative impact on the environment. Therefore, the multipurpose utilization of tea waste leaves needs to be developed and improved.

In this study, the solid-state fermentation of *G. frondosa* and *G. lucidum* (Tianzhi) by using the 4th to 7th tea leaves was studied, with the aim to develop a new tea processing technology that can transform tea waste leaves into a value-added new type of healthy tea product.

Material and Method

Tea Bush Species and its Leaves

The tea bush, cultivar Jinxuan was planted in South China Agricultural University. The 4th to 7th leaves plucked and used as substrate in this study.

Strain and Culture

Grifola frondosa and new variants of *Ganoderma lucidum,* namely Tianzhi, were cultured in a potato dextrose agar medium at 25 °C. After 7 days, the two strains were acclimated in a potato dextrose agar which added 3 % tea leaves powder.

Tea Substrate Preparation

The 4th to 7th tea leaves were collected, steamed to de-activate the enzymes, dried, crushed, and used as substrate. The acclimated strain was cultivated in the tea substrate at 25 °C.

Fermentation Conditions

The optimal levels of the three important factors were determined by response surface methodology.

The optimal incubation parameters for *G. frondosa* are summarized as follows: the main culture medium was the 4th to 7th tea leaves, the tea substrate was 35 %, the additional soybean powder was 3.49 %, pH was natural, fermentation temperature was 32.44 °C, fermentation time was 9 days, inoculation amount was 1 %, and water content was 65.10 %.

The optimal incubation parameters for Tianzhi are summarized as follows: the main culture medium was the 4th to 7th tea leaves, the tea substrate was 35 %, the additional soybean powder was 2 %, pH was natural, fermentation temperature was 25 °C, fermentation time was 16 days, inoculation amount was 4 %, and water content was 65 %.

Detection Methods for Active Components

Total content of polysaccharides was determined by the phenol–sulfuric acid method [15]. The protein concentration was measured according to Bradford's method [16] and the total phenols content was determined by Folin–Ciocalteu method [17]. The free amino acids were determined by the Ninhydrin method [18]. Caffeine was measured by high-performance liquid chromatography (HPLC) [19].

Polyphenols/Amino Acids Ratio Calculation

Tea polyphenols and amino acids are the major types of bioactive carbon and nitrogen compounds in tea leaves. The contents of the amino acids in tea have close relationships with tea freshness, appearance, sweetness, flavour and briskness degree. Meanwhile, the contents of polyphenols contribute largely to bitterness and astringentness. The ratio of tea polyphenols and amino acids reflects the match condition of the two quality components, representing an important indicator of the tea quality and mellowness [20].

The calculation formula of tea polyphenols/amino acids ratio (TP/A) was as follows:

TP/A = total contents of tea polyphenols/total contents of amino acids.

Amino Acid Detection Method

Sample preparation: 2 g of the fermentation tea substrate was placed in 50 mL flask and 20 mL of boiling distilled water was added. After 15 min, the tea infusion was collected through filtration with Whatman No. 4 filter paper, and the residue was infused by repeating this process for 4 times. The tea infusions were combined and distilled at 60 °C at a reduced pressure to obtain concentrated extraction. The concentrated solution was dissolved with 5 mL of 0.02 mol/L HCl and filtrated with 0.22 μm filter membrane.

The amino acid composition was determined using an amino acid automatic analyzer [21].

The detection conditions for amino acid were as follows: Chromatography column: 855–350 type; column temperature: programmed heating; reaction column temperature: 134 °C; analysis time: 110 min; sample size: 20 μL.

Volatile Constituents Detection Method

The volatile constituents were determined by gas chromatography-mass spectrometry (GC/MS) [22].

Sample preparation: The volatile constituents of the fermentation tea substrate were extracted with headspace solid-phase micro extraction. 10 g of the fermentation tea substrate was extracted in 150 mL vial equipped with a vacationer stopper and needle septum. The volatile constituents were obtained under the following conditions of stirring at 60°C for 30 min, using 75 μm PDMS/DVB coated fibre, then desorbing at 220 °C for 3 min.

The detection conditions for GC/MS were as follows:

Chromatography column: HP-INNOWAX, 30 × 0.25 mm × 1.0 μm.

Chromatographic conditions: the carrier gas was helium at a flow rate of 1 mL/min. The injector was kept at 220 °C. The oven temperature was programmed as follows: 60 °C for 2 min, 80 °C at 3 °C/min for 2 min, to 180 °C at 10 °C/min, kept for 2 min, to 220 °C at 2 °C/min for 5 min.

Mass spectrum conditions: ion source: electron ionization (EI); electric energy: 70 eV; electric tension: 350 V; scan range: 35–335 amu.

Sensory Evaluation

The sensory evaluation was carried out unto human volunteers to examine the effect of the brewing conditions (crushing degrees, the ratio of tea substrates and water, and brewing time) on the sensory quality of *G. frondosa* and Tianzhi fermented teas. The L_9 (3^4) orthogonal experimental design of the brewing conditions was shown in Table 1.

Statistical Analysis

Data are presented as mean ± standard deviation of triplicate measurements and were analyzed by SPSS 17.0 software. The Duncan's multiple range test was applied to determine the significance of differences ($P < 0.05$).

Results and Discussion

The Active Ingredients of the Fermented Tea Substrate

As shown in Table 2, significant differences were detected in total sugar, crude polysaccharides, tea polyphenols, amino acids, polyphenols/amino acids ratio, caffeine and water extracts. For total sugar, a statistically significant difference between *G. frondosa* and Tianzhi fermented teas was observed ($P < 0.05$). Their total sugar contents were 13.31 and 10.60 % respectively. Similarly, for the crude polysaccharides, a significant difference between *G. frondosa* and Tianzhi fermented teas was also observed ($P < 0.05$), and their contents were 5.49 and 4.14 % respectively. Amino acid contents were 1.52 and 0.94 %, and polyphenols/amino acids ratio were 1.00 and 1.98 respectively, with a significant difference ($P < 0.05$). The level of tea polyphenols in *G. frondosa*-fermented tea was 1.51 %, which was lower than that in Tianzhi-fermented tea (1.85 ± 0.227). Moreover, water extracts in *G. frondosa*-fermented tea was 35.53 %, which was higher than that in Tianzhi-fermented tea (32.86 %).

Table 1 The orthogonal experimental design of the brewing conditions

Level	Crushing degrees (μm)	Tea substrates/water ratio	Brewing time (min)
1	590	1:50	3
2	420	1:75	5
3	250	1:100	7

Free amino acids, tea polyphenols, caffeine, ascorbic acid, and other substances constitute the main flavor compounds of tea. Their contents and proportions are closely related to the taste of the tea. Free amino acids are the main contributor for the umami of tea. Tea polyphenols are the main components of astringency, and caffeine is the main component of the bitter taste. The amino acid contents and the polyphenols/amino acids ratio are indexes of tea quality. The TP/A ratio is a comprehensive response of taste compounds, which determines largely the taste of the tea. Namely, a higher ratio indicates a stronger astringency, whereas a lower ratio indicates a more bitter taste. In the present study, TP/A ratio was significantly decreased after fermentation with the two edible fungi. Their ratios in the *G. frondosa* and Tianzhi-fermented tea decreased by 88.28 and 76.79 %, respectively. Likewise, the content of water-soluble polysaccharides also determines the tea quality significantly. The content of polysaccharides in *G. frondosa*-fermented tea increased twice and the content of polysaccharides in Tianzhi-fermented tea increased 1.61 times. Compared with the widely consumed Oolong tea, the contents of polyphenols/amino acids ratio and caffeine in fungal fermented tea were significantly lowered, indicating a significantly reduced tea bitterness that may improve the taste.

Amino Acid Compositions of the Fermented Tea Substrates

The amino acid compositions of the fermented tea were shown in Table 3.

The *G. frondosa*-fermented tea contained 33 kinds of amino acids, with essential amino acids content of 35.15 %, Lys content of 490.60 mg/100 g, Arg content of 565.1 mg/100 g, and Glu content of 476.3 mg/100 g. The Tianzhi-fermented tea contained 30 kinds of amino

Table 2 Comparison of bioactive components in *G. frondosa*- and Tianzhi-fermented tea substrates

Components	*G. frondosa*	Tianzhi
Total saccharide (%)	13.31 ± 0.699[a]	10.60 ± 0.636[b]
Polysaccharides (%)	5.49 ± 0.129[a]	4.14 ± 0.372[b]
Tea polyphenols (%)	1.51 ± 0.200[a]	1.85 ± 0.227[a]
Amino acids (%)	1.52 ± 0.053[a]	0.94 ± 0.065[b]
Polyphenols/amino acids	1.00 ± 0.150[a]	1.98 ± 0.194[b]
Caffeine (%)	1.16 ± 0.021[a]	1.70 ± 0.236[b]
Water extraction (%)	35.53 ± 0.708[a]	32.86 ± 0.859[a]

Results are presented as means ± standard errors of triplicate measurements ($P < 0.05$)

[a, b] Mean significant difference ($P < 0.05$)

acids with essential amino acids content of 21.37 %, and total amino acid of about 1678.3 mg/100 g. Furthermore, the two fermented teas contained many non-protein amino acids, such as taurine, γ-aminobutyric acid, carnosine and so on. Taurine is an essential nutritive element for infant growth and development. It can strengthen night vision capabilities and immune system, improve the protective effect of the cardiovascular system and accelerate fat emulsification [23]. Γ-aminobutyric acid is a strong nerve inhibitory amino acid with sedation and hypnosis, anti-convulsive, and hypotensive functions [24]. Carnosine is a kind of natural antioxidant as well as an olfactory nerve sensor that was able to promote wound healing, especially during in surgical treatment [25].The contents of the non-protein amino acids taurine, γ-amino butyric acid and carnosine were 70.5, 156.8 and 115.8 mg/100 g in *G.frondosa* fermented tea; and 101.9, 58.1, 14.2 mg/100 g in Tianzhi-fermented tea, all were higher than those in common tea. Overall, the kinds and contents of amino acids in *G. frondosa* fermented tea were superior to those in Tianzhi fermented tea, except that in Tianzhi-fermented tea taurine content was 1.45 times higher than that in *G. frondosa*-fermented tea.

The Volatile Constituents in the Fermented Tea Substrates

The fermented tea was analyzed by GC/MS, and the result was shown in Table 4.

In *G. frondosa*-fermented tea, 32 volatile components were detected, including 1 alkyl hydrocarbon (2.74 %), 9 alcohols (21.39 %), 6 aldehydes (11.72 %), 3 ketones (4.54 %), 6 esters (18.81 %), 2 organic acids (6.64 %), and 5 others (9.42 %). The volatile components with the highest contents were 3, 4-dimethylcyclohexanol (2.47), 2,2,6-trimethyl-6-vinyltetranhydro-2H-pyran-3-ol (5.96), hexanal (4.74), valerolactone (2.05), ethyl caprate (7.99), ethyl laurate (3.71), isobutyricacid (2.05), isovaleric acid (4.15) and ortho-xylene (2.74), and *N*-ethylsuccinimide (3.46).

Table 3 Free amino acid composition in *G. frondosa*- and Tianzhi-fermented tea substrates (mg/100 g)

Amino acid	*G. frondosa*	Tianzhi
Phosphoserine	226.8	338.3
Taurine	70.5	101.9
Aspartic acid	125.5	92.1
Threonine	291.9	58.9
Serine	322.4	84.5
Glutamic acid	476.3	148.3
Theanine	21.6	2.3
α-Amino adipic acid	57.3	12.5
Glycine	91.4	58.5
Alanine	446.3	165.4
Citrulline	0.094	\
α-Amino butyrate	0.063	5.8
Ethanolamine	40.8	11.9
Hydroxylysine	19.8	6.7
Ornithine	60.9	11
Lysine	490.6	70.9
Histidine	102.1	9.8
Valine	206.4	77
Cystine	30.1	6.3
Methionine	63.6	2.5
Cystathionine	129.7	7.9
Lsoleucine	197.1	54.8
Leucine	437.2	83.1
Tyrosine	83.5	50.9
Phenylalanine	101	\
β-Alanine	60.2	3.5
β-Amino butyrate	5.8	2.4
γ-Amino butyrate	156.8	58.1
Tryptophan	36.6	11.5
Anserine	121.5	\
Carnosine	115.8	14.2
Arginine	565.1	79.6
Proline	20.6	47.7

\, means that its value has not yet been detected yet

The Tianzhi-fermented tea contained 35 volatile components, including 3 alkyl hydrocarbons (5.29 %), 5 alcohols (6.40 %), 9 aldehydes (20.63 %), 8 ketones (17.27 %), 3 esters (9.44 %), 1 organic acids (5.88 %), and 6 others (17.32 %). The volatile components with the highest content were aldehyde (3.03), 2-octenal (2.42), cis-6-nonenal (2.71), trans-2,cis-6-nonadienal (2.01), methyl heptenone (2.68), 2-cyclohexen-1-one (1.38), geranylacetone (3.90), citronellyl formate (1.73), ethyl caprate (3.38), allyl caproate (2.10), 2-caproic acid (4.49), 1,2-dimethylbenzene (3.35), and *N*-ethylsuccinimide (3.48).

Table 4 The volatile constituents in *G. frondosa*- and Tianzhi-fermented tea substrates

Retention time (min)	Names of constituents	Content (%)	
		G. frondosa	Tianzhi
4.60	Hexanal	4.74	3.03
5.59	1,2-Dimethylbenzene	2.74	3.35
11.42	6-Methylhept-5-en-2-one	1.23	2.68
13.05	Nonanal	1.75	1.86
13.2	Citronellyl formate	–	1.73
13.37	3-Octen-2-one	–	1.33
13.89	2-Octenal	0.92	2.42
14.06	Ethyl Octanoate	0.85	–
14.43	1-Octen-3-ol	1.47	1.3
14.63	2,4-Heptadienal	0.66	0.94
14.85	2-Furanmethanol,5-ethenyltetrahydro-a,a,5-trimethyl-, (2R,5R)-rel-	2.85	–
15.21	4-Ethylcyclohexanol	1.24	1.48
15.99	cis-6-Nonenal	1.11	2.71
16.14	1,3-Butanediol	0.7	–
16.39	1-Undecene,8-methyl	–	0.94
16.5	Isobutyricacid	2.05	–
16.56	3,5-Octadien-2-one, (E,E)	–	1.13
16.79	Ttrans-2, cis-6-Nonadienal	–	2.01
16.96	1,2-Propanediol	1.86	–
17.09	3,4-Dimethylcyclohexanol	2.47	0.89
17.16	1-Ethyl-1H-Pyrrole-2-carboxaldehyde	–	1.49
17.26	Valerolactone	2.05	–
17.34	2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde	1.76	0.94
17.56	Ethyl caprate	7.99	3.38
17.74	4-(Benzoyloxy)-2H-Pyran-3-one	1.62	0.95
17.91	Allyl caproate	–	2.1
18.00	3-Methylbutyric acid	4.15	–
18.37	2-Cyclohexen-1-one	–	1.38
18.73	1,2,4a,5,6,8a-Hexahydro-4,7-dimethyl-1- (1-methylethyl) naphthalene,	–	1.00
18.84	2, 3-Dimethylmaleic anhydride	–	0.99
18.97	Tricyclo (4.3.2.0(1,4))undeca-2,4(5), 8,10-tetranen-7-one	–	1.16
19.12	Oxime-methoxy-phenyl	2.12	–
19.19	Naphthalene,1,2,3,4,4a5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)	–	6.84
19.25	2,2,6-Trimethyl-6-vinyltetranhydro-2H-pyran-3-ol	5.96	–
19.42	Methyl salicylate	0.96	–
19.82	2,4-Decadienal	–	0.36
19.9	Phenylethyl acetate	1.99	–
20.14	Ethyl dodecanoate	3.71	–
20.16	Hexanoic acid	–	4.49
20.82	4-Methyl-2(5H)-furanone	–	0.4
20.92	2,6-Di-tert-butyl-4-methylphenol	–	0.19
20.31	2,10-Dimethyl-5,9-undecadien-2-one, Geranyl acetone	1.55	3.9
20.6	Benzyl alcohol	0.38	–
20.7	*N*-Ethylsuccinimide	3.46	3.48
20.93	Bornadiene	0.24	–
21.03	Phenethyl alcohol	2.68	1.03
21.39	4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	1.43	1.21
21.66	Benzothiazole	1.35	0.7
22.2	1-Methoxymethyl-4-methoxy-1-naphthol	–	0.27

–, means that its value has not been detected yet

Volatile components are one of the most important factors to affect flavour, taste and other quality parameters of tea [26]. After fermentation, the volatile components of the two kinds of fungus fermented teas were mainly composed of alcohols, esters, aldehydes and ketones. Compared with some reports about tea volatile components, there was an obvious difference between the fungi fermented tea and traditional tea. Gu et al. [27] reported that benzyl alcohol, linalool oxide, and linalool were greatly rich in Pu-erh raw teas, while the contents of 1,2,3-trimethoxylbenzene and 1,2,4-trimethoxylbenzene were much high in Pu-erh ripe teas. Jumtee et al. [28] had analyzed the volatile compounds of Japanese green tea (Sen-cha) which including geraniol, indole, linalool, *cis*-jasmone, dihydroactinidiolide, 6-chloroindole, methyl jasmonate, coumarin, trans-geranylacetone, linalool oxides, 5, 6-epoxy-β-ionone, phytol, and phenylethyl alcohol. Apparently, the fermentation of the edible fungi mycelia altered and enriched the composition of volatile components in the tea substrate.

Sensory Evaluation of the Fermented Tea

The results of sensory evaluation were shown in Tables 5 and 6.

Table 5 indicates that the best brewing conditions for *G. frondosa*-fermented tea were as follows: crushing degrees of 250 μm, tea substrates/water ratio of 1:75, and brewing time for 3 min. According to the range differences in Table 5, the influential degree of the three factors on sensory quality followed this order: tea substrates/water ratio >crushing degrees >brewing time.

Table 5 Range analysis of results in orthogonal test for sensory evaluation of *G. frondosa* fermented tea

Test number	A	B	C	Score
1	1	1	1	75.00
2	1	2	2	75.00
3	1	3	3	82.61
4	2	1	2	77.67
5	2	2	3	77.00
6	2	3	1	83.33
7	3	1	3	77.67
8	3	2	1	85.00
9	3	3	2	82.00
K1	77.537	76.780	81.110	
K2	79.333	79.000	78.223	
K3	81.557	82.647	79.093	
R	4.020	5.867	2.887	

K1, K2, K3 represent the averages of the three factors

R means range analysis

Table 6 Range analysis of results in orthogonal test for sensory evaluation of Tianzhi fermented tea

Test number	A	B	C	Score
1	1	1	1	89.43
2	1	2	2	75.76
3	1	3	3	82.67
4	2	1	2	77.67
5	2	2	3	77.00
6	2	3	1	90.00
7	3	1	3	77.67
8	3	2	1	88.45
9	3	3	2	82.00
K1	82.620	81.590	89.293	
K2	81.557	80.403	78.447	
K3	82.707	84.890	79.113	
R	1.150	4.487	10.816	

K1, K2, K3 represent the averages of the three factors

R means range analysis

As shown in Table 6, the best brewing conditions for Tianzhi-fermented tea was as follows: crushing degrees of 420 μm, tea substrates/water ratio of 1:100, and brewing time for 3 min. The influential degree of the three factors on sensory quality followed this order: brewing time >tea substrates/water ratio >crushing degrees.

To combine the results of Table 2 and sensory evaluation, we found that the polysaccharides, free amino acids, and protein contents were significantly improved, and the fungal fermented tea tasted more fresh, crisp and mellow after fermentation. In addition, tea polyphenols, TP/A, caffeine, and water extract contents in the fermented products significantly decreased which weakened the molecular association interaction, decreased the tea juice turbidity, reduced bitterness, and had a sweet taste and aroma.

By fermentation with the medicinal fungi, the low-grade tea leaves were significantly upgraded, and a product with enriched beneficial, bioactive compounds from fungal metabolism as well as from tea itself was obtained. In addition, the flavor of the fermented tea was also improved, which indicated good consumer acceptance and a promising market prospect.

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