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Journal of Food Science & Technology (ISSN: 2472-6419)

Characteristics of Keemun black tea, dark tea and green tea processed from *C. sinensis var.* Zhuye

DOI: 10.25177/JFST.5.3.RA.10641

Research

Accepted Date: 25th April 2020; Published Date:04th May 2020

Zhenmin Hu[1], Zeyi Ai[1], Jianhua Yang[2], Hao Zheng[2], Zhengtao Chen[3], Haihua Shi[3]

[1]Institute of leisure agriculture, Jiangsu academy of agricultural sciences, Nanjing, Jiangsu province, P. R. China

[2]Suzhou Xinfeng modern agricultural plantation, Suzhou , P. R. China

[3]Jiangsu Liyang Yulian ecology agricultural developing com. Ltd. Changzhou, Jiangsu province, P. R. China

Zhenmin Hu: 295064802@qq.com; Zeyi Ai: 147009774@qq.com; Jianhua Yang: 12376158@qq.com;

Hao Zheng: 1123225240@qq.com; Haihua Shi: 40620431@ qq.com;

CITATION

Zhenmin Hu, Zeyi Ai, Jianhua Yang, Hao Zheng, Zhengtao Chen, Haihua Shi, Characteristics of Keemun black tea, dark tea and green tea processed from C. sinensis var. Zhuye(2020)Journal of Food Science & Technology 5(3) pp:111-124

ABSTRACT

Sensor quality, chemical components as well as radical scavenging ability of 3 kinds of tea, namely, spiral-shaped black tea, Ancha dark tea, Maofeng green tea, which are processed from Camellia sinensis, cultivar Zhuye, in Keemun tea growing area, were investigated. The taste of the dark tea could be described as heavy and mellow, but it possesses neither stale taste nor aging aroma. The content of epigallocatechin gallate (EGCG) in green tea was the highest in all 3 kinds of tested tea, while the contents of epigallocatechin (EGC) and gallocatechin gallate (GCG) in the dark tea was higher than that in Green tea. The typical aroma component linalool oxide and geraniol reached 8% and 14.5% of the total essential oils content in the black tea, respectively. The roast fragrance components 6-ethenyltetrahydro-2,2,6-trimethyl-2H-pyran-3-ol and 1-ethyl-1H -pyrrole-2-carboxaldehyde were also found in the spiral-shaped black tea, which may be produced in the roasting stage of the shaping process.

Difference of the scavenging effect for free radical DPPH, superoxide anionic and hydroxyl radical between the 3 kinds of tea was not significant, while the general reducing power was green tea > dark tea > black tea. There was no common trend for the impact of extracting methods for different radical scavenging assays for the tested tea samples.

Keywords: quality, anti-oxidation, spiral-shaped black tea, Ancha dark tea, Maofeng green tea, Keemun

1. INTRODUCTION

Since acquiring its first gold medal in *Panama* Pacific International Exposition in 1915, Keemun *Congou* black tea has become famous as one of the highest fragrant black tea in the world. However, a kind of dark tea called *Ancha* with special flavor and health-promoting characters, is also produced in this area (Huang et al., 2009; Ning et al., 2016). In addition, green tea processed in this traditional black tea producing area is also of excellent quality.

Dark tea is one of the 6 categories of tea produced in China, its processing steps include de-enzyme, rolling, post-fermentation, drying and so on. With a de-enzyme processing step at first, there is no strong enzyme-catalysis oxidation of catechins in its post-fermentation, while auto-oxidation or the microorganism action play an important role in its processing. On the other hand in the processing of black tea the first step is withering, in which the enzymes of polyphenol oxidase and peroxidase is highly activated, the catechins oxidation during its fermentation is catalyzed mainly by oxidases.

The famous Keemun black tea is traditionally in the shape of strip, however, to meet the demand of consumers for the special shape of this famous tea, a kind of spiral-shaped Keemun black tea named *red fragrance spiral* was created (Fig. 1). The special flavor and the elegant shape of spiral, similar to the famous Chinese green tea *Dongting Biluochun* (produced in Suzhou, Jiangsu province) has made it more popular in both domestic and international markets.

Fig 1. Various Keemun tea products

Despite being famous for over a century in China, Keemun (*Ancha*) dark tea is rarely known internationally. With the unique quality character of the tea (*Camellia Sinensis*) cultivar *Zhuye* and the special processing schedule, *Ancha* presents the special character of high fragrance and delicious taste. *Ancha* was first produced in Ming dynasty and became very popular before the famous Keemun black tea was created (Huang et al., 2009; Geng, 2014; Ning et al., 2016). During the 1930s-40s, its market disappeared, due to war, and thus its processing technology was lost. It was not until 1984 that the technicians in Keemun county recovered its processing technology based on the samples which were stored in Hong Kong (Geng, 2014; Ning et al., 2016).

It is well known that green tea is the most favored tea category for Chinese consumers and can benefit the tea producers most. Now in the traditional Keemun black tea producing area, about a quarter of the tea product is green tea, which is called Keemun *Maofeng* named after its appearance covered by thick hair, just like the famous Chinese tea Huangshan *Maofeng*. However, the fresh shoots come from the same tea plant cultivar *Zhuye* as Keemun black tea.

Not only its pleasant flavor and elegant shape, the health function is also the most important reason that tea become an excellent non-alcohol drink in the world. The anti-oxidant function of tea is an important index reflecting the tea health-promoting property. Free radicals accompanying with the abnor-

mal oxidation in cell can attribute to a lot of human diseases for the physiological reasons, such as cancer, inflammation, diabetes, cardiovascular disease and so on (Nanjo et al., 1999, Yang et al., 2018). Considering of these, the free radical scavenging abilities as well as the anti-oxidant function of tea is one of the most important attributes for its consumption.

Up to now the quality analysis on black tea is usually focused on the traditional Keemun *Congou* black tea, and some reports have revealed the anti-oxidant function of black tea (Wang et al., 2018). However, little work has been done on the new Keemun tea products. In this study the chemical components as well as the anti-oxidation function were investigated for the 3 kinds of Keemun tea, namely spiral-shaped Keemun black tea, Keemun dark tea (*Ancha*) and Keemun green tea (*Maofeng*).

It was ever reported that both the water soluble extracts and the residues of tea have the anti-oxidant abilities, and it is believed that the extract method has an impact on antioxidant efficacy(Claudia et al., 2015) . Therefore, in this study two different solvent were used to get the extracts and their anti-oxidant abilities were also analyzed, respectively.

2. Materials and methods

2.1 Tea samples

The 3 kinds of tea samples were prepared in Keemun are the spiral-shaped Keemun black tea, Keemun dark tea, and Keemun green tea. Black tea and green tea were processed in April of 2017. For dark tea the harvested leaves were processed firstly to a drying tea material in April of 2017 and the final sample was made in November of 2017.

The main processing schedule for the 3 kinds of tea is in brief as follows:

Spiral-shaped Keemun black tea: fresh shoots was spread on bamboo sheet and withered naturally at 22℃-25℃ for 12hr, and then rolled for 30min and ferment for 4hr at 30℃ and RH 90% . The fermented leaves were dried in a chain plate dryer at 120℃ for 5min, and then rolled and rubbed at a pot until the tea acquire the spiral shape. Finally, the semi-finished tea was dried at 110℃ for 15 min.

Keemun green tea: fresh shoots were first fixed (deenzyme) at a *cylinder* pot at 220℃ for 8min, then rolled for 10min in a rolling machine, and finally dried at 110℃ for 20 min followed by 100℃ at 15 min in a dryer.

Keemun dark tea: fresh shoots were first fixed (enzyme deactivated) in a *cylinder* pot at 220℃ for 8min, and then rolled for 45min with machine, after that dried at 110℃ and 100℃ for 20 min and 15 min, respectively. The dried tea was stored at room temperature for 5-6 months as the primary material for dark tea. After then the primary material was exposed under the sky over-night (called dewing tea). The dewed tea was steamed by water vapour for 3-5 min and cooled. Then the steamed tea was weighted into a special bamboo basket, pressured by hand, and was then dried with charcoal fire for 48h in a cotton covered manner. The finally dried tea was the Keemun dark tea.

All of the made tea were stored at 4 ℃ for the following analysis.

2.2 Major reagents and facilities

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) and authentic standard catechins were purchased from Sigma-ldrich Shanghai trading Co. Ltd. (Shanghai, China). The other chemicals were purchased from Shanghai chemicals Co. Ltd. (Shanghai, China).

All colorimetric measurements were determined using UV-3600 spectrophotometer (Shimadzu, Kyoto, Japan). For HPLC analysis, Agilent 1100 (Palo Alto, CA,USA) was used. Hitachi automatic amino acid analyzer (L-8900) was used to measure the content of individual amino acids.

2.3 Sensory evaluation

Based on the procedure of China national standard GB/T 23776 “Methodology for Sensory Evaluation of Tea”, 5 experts were employed to evaluate the samples and gave description of the characteristics for each tea sample.

2.4 Analysis of major chemical constituents

2.4.1 Tea polyphenols and the composition of catechins

Based on the method of China national standard GB/T 8313-2008, and modified according to the reports (Masoomeh et al., 2008), the total polyphenol content was determined using the Folin-Ciocâlteu method with the gallic acid (GA) equivalent as standard.

HPLC system equipped with a Shimpack VP-ODS C18 column (5mm, 4.6×150mm) was employed for catechins and caffeine analysis. Solvents A (water) and B (N, N-dimethylformamide/ methanol/ acetic acid, 20:1:0.5, v/v/v) were run in linear gradients with B increasing from 14% to 23% within 13min, from 23% to 36% within next 12min and maintained for 3min thereafter at a rate of 1.0mL min^{-1}. The content of catechins as well as caffeine were quantified by their peak areas against those of standards prepared from authentic standard (Sigma-ldrich Shanghai trading Co. Ltd. (Shanghai, China)).

2.4.2 Composition of amino acids

Composition of amino acids was determined using Hitachi automatic amino acid analyzer(L-8900) based on the schedule as described in the literature (Tapuhi et al., 1981).

2.4.3 Analysis of volatile compounds

Method described by Ershad Sheibani (Ershad S. et al., 2016) with slight modification was used to analyze the volatile compounds , SPME method was employed with guaiacol as internal standard.

One gram of tea sample was placed into a glass tube and 50mL boiling water was added to the tube. Then the cap of the tube was screwed and kept at a water bath at 70℃ for 10 min. The resultant solutes were filtered with 4 layers of gauze and cooled.

The filtered solute was stored at other glass tube. A 10mL filtered tea solutes was put into the SPME extracting bottle, 20μL guaiacol internal standard was added to the bottle, and vortexed for 30s, then 2g dry NaCl was added. The cap of the bottle was screwed and the bottle was vortexed till the NaCl dissolved completely.

The extraction head (50/30 μm DVB/CAR/PDMS） was pierced into the extracting bottle for head space extraction and the bottle was cubed at a water bath of 40℃ for 1h. After then the extraction head was plugged into the GC-MS sampler equipment, de-absorption for 3.5min.

GC/MS condition: Instrument GC/MS QP-2010 Ultra (Shimadzu, Japan). GC: Chromatographic column HP-Innowax (30m*0.25mmID*0.25um film thickness), The oven temperature was programmed as follows: the initial temperature was held at 50 °C for 5 min, and the column was then heated at 3 °C/min to 210 °C and held for 3 min before being heated at 15 °C/min to 230 °C and finally to 250 °C. Helium (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min.

The mass spectra were collected in the EI ionization mode, with an electron energy of 70eV, interface temperature of 280 °C, ion source temperature of 230 °C, quadrupole temperature of 150 °C, scanning range of 35–400 amu, and an emission current of 34.6 μA. Qualitative identified: National Institute of Standards and Technology (NIST) mass spectrometry library. Quantitative analysis: the concentrations of volatile compounds are expressed as ratios of candidate material peak area / inner peak area.

All of the samples were analyzed in triplicate (independent experiment).

3. Antioxidant test

3.1 Extraction of samples

Samples were extracted with hot water and acid-methanol respectively.

For water extracting, 0.5 g samples were suspended with 40 mL boiling deionized water in centrifuge tubes, then thoroughly shaken at 75 °C water bath for 30 min. The mixture was separated by centrifuge at 5000g for 10 min, the supernatant was collected. The residue was extract with another 30 mL water at 75 °C water bath for 20 min and centrifuge, the supernatant of the two steps was merged and adjusted to a

final volume of 100 mL with deionized water and stored at 4 °C prior to further analysis.
For acid-extraction, 75% (v/v) methanol containing 0.1% formic acid (v/v) was used as solution, and the other steps are the same as water extracting method.

3.2 DPPH radical scavenging activity assay
The scavenging activity against DPPH of tea was evaluated by the method of literature (Brand et al., 1995) after slight adjustment.

Method in brief was as follows: DPPH about 40mg/L was adjusted by ethanol to the concentration that its absorption at 517 nm was between 0.600-0.700.

For background absorbance of DPPH, 1.0mL ethanol mixed with 2.5mL DPPH solution, for tea infusion background absorbance, 1.0mL tea infusion mixed with 2.5mL ethanol. In the radical scavenging test tube, 2.5mL DPPH solution mixed with 1.0mL tea infusion. All tubes were kept at room temperature for 0.5 h, then the absorbance of 517 nm was measured. Radical scavenging activity was expressed as scavenging percentage and was calculated as follows:

DPPH scavenging activity (%) = $(1-(Ax-A_{X0}) /A_0) \times 100\%$

where A_0 is the absorbance of the free DPPH, Ax_0 is the absorbance of the tea infusion as background and Ax is the absorbance of radical scavenging system.

3.2.2 Hydroxyl radical (• OH) scavenging assay
This assay was based on the method established by Halliwell B. and Gutteridge J.M.C. (Halliwell et al., 1984) with slight adjustment.

In a 10mL PET tube 1 mL of 6 mmol/L ammonium ferrous sulfate ($Fe(NH_4)_2(SO_4)_2$) , 1mL 6 mmol /L salicylic acid (SA) and 1 mL tea infusion were mixed and kept at room temperature for 30 min , and then 1mL 8mM H_2O_2 was added to. 5min later, the absorbance Ax was determined at 510 nm. Absorbance of A_0 (with no tea soup in the reaction system), and Ax_0 (H_2O_2 was replaced by water) were determined at the same time.

• OH scavenging activity (%) = $(1-(Ax-A_{X0}) /A_0) \times 100\%$

3.2.3 Superoxide radical scavenging assay
The superoxide radical scavenging activity was evaluated by monitoring the inhibition of pyrogallol autoxidation (Chen et al., 2015). Tea sample was mixed with 0.1 mol/L, pH 7.5 tris–HCl buffer and 30 mmol/L pyrogallol solutions, vibrated rapidly. The mixture was keep at 25℃ water bath for 10min, and then 0.5mL 0.1 mol/L HCl was added to terminate the reaction, absorbance of 420 nm (A_x) was measured, the background absorption (A_0, A_{X0}) of the tea soup and pyrogallol solutions were all measured at 420 nm at the same time.

Radical scavenging activity was calculated as following:
O^-_2 · scavenging activity (%) = $(A_X-((A_0-A_{X0}) / A_0) \times 100\%$
where A_0 is the absorbance of the control pyrogallol solutions (with no tea infusion in the system), A_{x0} is the absorbance of tea infusion (with no pyrogallol solutions in the system).

3.2.4 Reducing power assay
The reducing power was measured by ascorbic acid method (Aisaka et al., 1978).
The first order reaction system was as follows: 1mL tea soup was mixed with 2.5mL phosphate buffer (0.2 mol/L, pH 6.6) and 2.5mL potassium ferricyanide (1%, w/v). The mixture was kept at water bath of 50℃for 20 min and then cooled rapidly. After that 2.5mL of trichloroacetic acid (10%, w/v) was added and the mixture was centrifuged at 2797.5g for 10 min. 2.5 mL of the supernatant was mixed with 2.0 mL of distilled water and 0.5 mL of ferric chloride (0.1%, w/v) to active the second reaction system. After 10 min, the absorbance of the second reaction system was measured at 700 nm.

For regent blank test, except that in the second reaction system 0.5 mL dilute water was added instead of the ferric chloride, all the other steps were the same to the sample test.

Ascorbic acid (Vc) was used as a reference material of reducing power assay, where 1mL of 0.1, 0.2, 0.3, 0.5, 0.8, 1.0 mg/L Vc were tested to get a reducing power curve to value the equivalent of the tea samples.

3.2.5 Statistical analysis

All the experiments were performed in triplicate and centered. Analysis of variance was performed by SPSS 19.0 and Excel.

4. RESULTS

4.1. Sensory characteristics

Table 1. Sensory description of the 3 kinds of tea

	appear-ance	liquor color	aroma	taste
black tea	wiry, black-bloomed	brilliant red	fragrance, persistent	sweet and brisk
dark tea	blackish-auburn	bright red	chestnut flavor	heavy and mellow
green tea	vivid green, even	bright yellowish-green	fresh	fresh and mellow

Among the three kinds of tea, Keemun black tea and green tea got the characteristics of the typical black tea and green tea respectively, while the taste of dark tea was described as heavy and mellow. This suggests that the infusion of the dark tea was rich and abundant in soluble substance while not harsh and astringent. In addition, the Keemun dark tea, *Ancha* no stale taste or aging aroma was noted.

4.2. Main chemicals analysis results

Table 2. Polyphenols and caffeine content in tea samples (%)

	polyphenols (water extracted)	polyphenols (e (ethanol extracted)	caffeine	TF
black tea	10.70±0.22a	12.58±0.76a	4.02±0.07a	1.539±0.11a
dark tea	16.58±0.15b	21 28±0.57b	3.70±0.11a	0.279±0.33b
green tea	22.18±0.40c	24.72±0.48b	3.83±0.67a	

For ethanol extracting, the phenol extracting capacity was 11.45%-28.34% more than that of water extracting. Caffeine content in black tea was higher than that in dark tea and green tea, but there was no significant difference between them.

Table 3. Catechins composition in tea samples (mg/g)

	GC	EGC	C	EC	EGCG	GCG	ECG	CG	total
Black tea	1.41±0.00a	8.84±0.02a	0.04±0.00a	1.24±0.01a	3.72±0.00a	1.31±0.06a	2.67±0.11a	1.25±0.07a	23.49±0.23a
Dark tea	3.37±0.10b	26.24±0.16b	0.88±0.01b	5.46±0.04b	8.81±0.42b	7.04±0.06b	13.68±0.05b	3.45±0.21b	118.93±0.63b
Green tea	0.14±0.04c	17.04±0.25c	1.09±0.02c	5.64±0.13b	18.33±3.01c	1.12±0.01a	32.17±0.90c	3.79±0.15b	179.31±4.42c

Content of the total catechins in the 3 kinds of tea showed that green tea was the highest. EGCG content in dark tea and black tea was 48.06% and 20.3% of that in green tea respectively, and among the other catechins, EGC, GCG content in dark tea was higher than that in green tea, while EC, CG content was similar between the dark tea and green tea.

Total amino acid content in dark tea and black tea was nearly the same, but much lower than that in green tea. For individual amino acids, theanine content in 3 kinds of tea was similar, and the content of the Thr, Glu, Arg in geen tea was about 2 times of that in dark tea and black tea. Except for theanine, content of threonine was the highest in all the 3 samples.

Table 4. Amino acids contents in the samples (%)

	black tea	dark tea		green tea
Thea	1.11	1.05		1.31
Asp	0.050	0.117		0.162
Thr	0.495	0.627		1.294
Ser	0.056	0.037		0.045
Glu	0.093	0.109		0.347
Gly	0.001	0.003		0.005
Ala	0.044	0.040		0.027
Cys	0.007	0.008		0.011
Val	0.029	0.024		0.017
Met	0.016	0.000		0.001
Ile	0.023	0.018		0.010
Leu	0.026	0.020		0.019
Tyr	0.053	0.021		0.022
Phe	0.058	0.039		0.031
Lys	0.020	0.030		0.027
His	0.061	0.046		0.022
Arg	0.059	0.090		0.194
Pro	0.031	0.010		0.000
total	2.235	2.294		3.565

Table 5. Aroma analysis data (relative area)

Compounds	black tea	dark tea	green tea
Dimethyl sulfide	0.00±0.00	0.00±0.00	1.22±0.30
2,4-Dimethyl-1-heptene	0.09±0.13	0.48±0.67	0.05±0.03
Butanal, 2-methyl-	2.07±0.17	0.96±1.36	0.26±0.37
Butanal, 3-methyl-	0.44±0.63	1.60±0.14	0.62±0.40
Pentanal	1.07±0.16	0.70±0.12	1.35±0.10
Toluene	0.56±0.30	0.30±0.14	0.15±0.22
Hexanal	6.49±1.38	0.54±0.21	1.08±0.04
3-Penten-2-one, 4-methyl-	0.00±0.00	0.31±0.03	0.00±0.00
1H-Pyrrole, 1-ethyl-	0.00±0.00	2.15±0.84	0.00±0.00
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	0.00±0.00	0.18±0.08	0.00±0.00
2-Heptenal, (Z)-	0.00±0.00	0.00±0.00	1.19±0.06
5-Hepten-2-one, 6-methyl-	0.73±0.15	0.30±0.09	0.09±0.13
1-Hexanol	0.53±0.10	0.05±0.07	0.26±0.02
Nonanal	0.14±0.20	0.14±0.20	0.29±0.41
Pyrazine, 2-ethyl-6-methyl-	0.00±0.00	0.25±0.07	0.00±0.00
3-Hexen-1-ol, (Z)-	0.91±0.23	0.33±0.46	0.45±0.64
trans-Linalool oxide (furanoid)	3.23±0.83	0.66±0.18	0.22±0.31
Pyrazine, 3-ethyl-2,5-dimethyl-	0.00±0.00	0.64±0.21	0.00±0.00
cis-Linalool oxide (furanoid)	5.74±1.38	1.12±0.32	0.58±0.13
2,4-Heptadienal, (E,E)-	1.29±0.23	0.00±0.00	0.00±0.00
1-Hexanol, 2-ethyl-	1.11±0.05	0.56±0.04	0.85±0.02
Benzaldehyde	6.63±5.87	2.96±0.12	2.46±0.14
1,6-Octadien-3-ol, 3,7-dimethyl-	7.62±1.23	2.01±0.22	3.56±0.03
1-Octanol	0.92±0.15	0.18±0.26	0.62±0.04
1H-Pyrrole-2-carboxaldehyde, 1-ethyl-	0.67±0.17	4.57±0.37	0.19±0.01
1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	0.42±0.08	0.10±0.15	0.00±0.00
1,5,7-Octatrien-3-ol, 3,7-dimethyl-	2.25±0.41	3.70±0.23	0.09±0.13
2-Octen-1-ol, (E)-	0.00±0.00	0.38±0.03	0.13±0.19
1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-	0.43±0.04	0.04±0.06	0.00±0.00
Acetophenone	0.00±0.00	0.27±0.03	0.00±0.00
1-Nonanol	0.36±0.10	0.12±0.03	0.18±0.00
1-Heptanol, 2-propyl-	0.54±0.20	0.63±0.10	0.31±0.03
L-.alpha.-Terpineol	0.21±0.30	0.38±0.12	0.00±0.00
2,6-Octadienal, 3,7-dimethyl-, (E)-	0.85±0.31	0.14±0.20	0.39±0.05
2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl	0.28±0.09	0.04±0.06	0.00±0.00
Methyl salicylate	3.18±0.50	1.48±0.18	1.63±0.02
Benzaldehyde, 2,4-dimethyl-	31.75±3.88	19.06±7.83	13.78±0.94
Irone	0.14±0.20	0.12±0.01	0.00±0.00
Geraniol	16.02±2.69	2.98±0.46	4.04±0.56
Phenol,2-methoxy-(Internal standard)	1.00±0.00	1.00±0.00	1.00±0.00
Benzyl alcohol	0.62±0.16	2.22±1.21	0.39±0.28
Phenylethyl Alcohol	2.48±0.31	0.66±0.08	0.74±0.05
trans-.beta.-Ionone	1.06±0.41	0.69±0.08	0.00±0.00

2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-	0.54±0.19	0.00±0.00	0.00±0.00
3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	0.38±0.12	0.08±0.12	0.00±0.00
Isopropyl myristate	0.17±0.24	0.21±0.05	0.05±0.07
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	0.39±0.04	0.08±0.11	0.04±0.05
Octanoic acid	0.42±0.06	0.16±0.00	0.12±0.01
1-Undecene, 8-methyl-	0.37±0.02	0.00±0.00	0.00±0.00
1,8(2H,5H)-Naphthalenedione, hexahydro-8a-methyl-, cis-	0.47±0.07	0.46±0.00	0.34±0.03
Nonanoic acid	2.09±0.41	0.00±0.00	0.00±0.00
Phenol,2-methoxy-4-(2-propenyl)-, acetate	1.03±0.04	1.55±0.05	0.00±0.00
2-Heptadecanone	0.00±0.00	0.30±0.04	0.07±0.10
Ethanol, 2-(dodecyloxy)-	0.08±0.11	0.34±0.04	0.13±0.18
n-Decanoic acid	1.24±0.76	0.86±0.12	0.50±0.08
2-Ethylhexyl salicylate	1.15±0.46	0.82±0.28	0.23±0.32
Homosalate	1.12±0.14	1.33±0.47	0.63±0.89
Dodecanoic acid	1.69±0.37	4.43±1.79	2.21±0.06
total	111.60±23.53	64.60±12.31	41.51±0.76

Note: The sum of total essential oil did not include the internal standard 2-methoxy-phenol

As showed in table 5, trans- and cis-linalool oxide as the prominent aroma component in Keemun black tea reached 8% of the total essential oil, and geraniol, another typical aroma component marked in the Keemun flavor reached 14.5% of the total essential oil in black tea.

Methyl salicylate, 2,4-dimethyl-benzaldehyde, phenylethyl alcohol, and benzaldehyde all reached a relative high level in the 3 kinds of Keemun tea, suggesting that they, together with geraniol, cis-linalool oxides and cis-3-hexenol, may be used to distinguish the special Keemun cultivar flavor.

4.3 Anti-oxidant assay results

Table 6. DPPH radical scavenging effect (%)

	water extracting	ethanol extracting
black tea	71.47±2.55a	83.55±1.12a
dark tea	74.82±4.03a	84.38±1.24a
green tea	70.52±2.99a	76.93±0.71a

For DPPH radical scavenging ability, the data showed that both in the water extracting group or the ethanol extracting group the dark tea was the most efficient. The difference was not significant within each group, but for each kind of tea the scanning effect between the two extracting methods was much different, with the radical scanning effect of ethanol extracting higher than that of the water extracting.

Table 7. Superoxide radical anion scavenging effect(%)

	water extracting	ethanol extracting
black tea	69.63 ± 9.67a	76.95 ± 8.20a
dark tea	77.95 ± 19.89b	91.83 ± 14.22b
green tea	71.23 ± 10.28a	90.68 ± 3.20b

Superoxide anionic radicals scavenging abilities assay results showed that both of the water extracts and the ethanol extracts of dark tea has a higher efficiency than that of green tea and black tea, and the ethanol extracting group has a stronger ability for superoxide anionic radicals scavenging than the water extracting group.

Table 8. Hydroxyl radicals scavenging effect (%)

	water extracting	ethanol extracting
black tea	51.20 ± 5.01b	66.81 ±3.63b
dark tea	65.06 ±1.96a	50.56 ±1.85a
green tea	71.23 ±0.27a	46.30 ±0.79a

Hydroxyl radicals scavenging assay results indicated that in the water extracting group the radicals scavenging effect was green tea > dark tea > black tea, but in the ethanol extracting group the radicals scavenging effect was black tea > dark tea > green tea, suggesting that the ethanol extracting method for black tea was more effective for hydroxyl radicals scavenging.

4.4 Reducing power assay

Reducing power curve of ascorbic acid (vitamin C, Vc) was as following:

$Y= -0.1031+0.9652X$ ($R^2 = 0.9949$)

Y: reducing power, the equivalent of Vc, mg/mL; X: O.D.420

Table 9. Reducing power assay for 3 kinds of tea (Vc equivalents)

	water extracting	ethanol extracting
black tea	0.488±0.026a	0.482±0.005a
dark tea	0.689±0.126b	0.654±0.036b
green tea	0.991±0.179c	1.086±0.192c

Evaluating with the ascorbic acid (Vc) equivalents it showed that both in the water extracting and the ethanol extracting group the reducing power of green tea was the highest, in both groups the ascorbic acid equivalent of green tea was double of that of the black tea. Yet comparing the data of water extracting and ethanol extracting, for each kind of tea the reducing power between the 2 groups had no significant difference.

5. DISCUSSION

5.1 Quality evaluation

It is believed that the special tea breeds, the soil and geology condition and the microclimate all have impact on the tea savory (Aisaka et al., 1978; Owuor et al., 2008), but the special processing method may benefit the quality most.

Our work showed the spiral-shaped black tea which involved a new method of roasting could also get a high fragrance, but there was a style of roasting flavor in it. Obviously there would be some components which marked the roasting fragrance. Aroma components 6-methyl-5-hepten-2-one, 6-ethenyltetrahydro-2,2,6-trimethyl-2H-Pyran-3-ol (Table 5) that associated with the roasting flavor was found to be rich in the spiral-shaped black tea sample, but they are rarely found in other reports on Keemun black tea (He et al., 2015, Wang et al., 2016). Nonanol, 2-heptanone and 3 -methylbutanal which was also considered to be associated with the roasting flavor (Yin et al., 2018; Xiao et al., 2017) help to develop the characters of black tea of the new style compared with the traditional *Gongou* black tea (Table 5).

Ancha, with its post-fermenting processing schedule which is to some extent similar to that of the typical dark tea is also defined as a kind of dark tea (Geng, 2014; Ning et al., 2016). In spite of this, its flavor and taste is obviously different from other dark teas. Sensory evaluation showed that there is neither stale smell nor mycelium smell in Keemun dark tea as that exists in Puer tea or Fuzhuan tea (Wang et al., 2016). Its infusion tastes stronger and smells a chestnut aroma with a little flower fragrance, yet the former is usually only found in green tea, and the latter is the typical character of Keemun flavor. These make its taste in general refresh and brisk (Table 3).

The difference between the *Ancha* and other dark teas is firstly attributed to fresh leaves for *Ancha,* which were relative tender, one bud and two or three leaves rather than five or six leaves, even stems was used. The second is to its post-fermenting processing during which there is no the wet and obvious microorganism reaction as for the other dark teas so that its

flavor has somewhat the characters of the interaction of black tea and green tea. Through slow oxidation of catechins by oxygen in air rather than by microorganisms during the storing and post-fermenting course, there is no stale taste formed in its quality. The special processing schedule of dewing tea and covering with cotton during drying process is also believed important for its flavor development, but the mechanism is still to be explored (Huang et al., 2009, Ning et al., 2016).

Amino acids are very important for tea quality (Liu et al.,2018). The big difference of amino acids in the 3 kinds of tea was attributed to the balance of protein hydrolyzation and amino acids oxidization during the processing. Though most of the individual amino acids content was the highest in green tea, several amino acids such as Ala, Val, Ile, Phe, His and Pro in the black tea and the dark tea were higher than that in the green tea. They contribute much to the taste of black tea and dark tea (Narukawa et al., 2014**)**. Theanine is not a protein constitute amino acid and may not be easy to oxidize, and thus is not significantly different between the 3 kinds of tea.

Glu and Asp, the mainly contribute composition to fresh taste were 0.307% and 0.167% in green tea and was the most in the 3 kinds of tea, that was coincide with the sensor evaluation result. Content of Glu in the dark tea and the black tea was nearly the same, though the Asp content in dark tea was about 2 times of that of black tea, because the taste threshold of Asp is higher than Glu, in general there was no great impact on the taste of the 2 kinds of ferment tea.

Sweet taste amino acids Gly, Ala, Ser and Pro in green tea in sum was 2.31% of the total amino acids, that is much lower than that of the dark tea and the black tea, combined with the high content of catechins gave green tea the brisk taste, while the high content of sweet amino acids of 6.14% of the total amino acids in black tea contributed much to the sweet taste of black tea.

It was worthy to note that the bitter amino acids Tyr, Phe, Leu, Ile, Val, Thr, His, Lys and Arg in sum in green tea was 43.20% of the total amino acids and much higher than that of 35.58% for black tea and 37.65% for dark tea. We usually attribute the astringency and bitter flavor of tea infusion to the polyphenols or saponin (Wang & Ruan，2009), according to this study, bitter amino acids may have a great impact on the astringency of green tea.

Total plyphenols content in the 3 samples was in accordance to our knowledge as green tea> dark tea> black tea, yet the high level of GC, EGC, GCG in dark tea was not expected. Ning et al. has studied the composition of catechins in Keemun dark tea (*Ancha*) (Ning et al., 2016), their results were similar to ours. It indicated that the oxidation of catechins in Keemun dark tea was less strong than in other dark teas, Lv has checked the catechins in Pu-erh tea, Fuzhuan tea and Liubao tea, and found no EGC and C in all the samples, content of EGCG in Pu-erh, Fuzhuan and Liubao tea was 0.23, 2.03, 1.19 mg/g respectively (Lv et al., 2017), and was much lower than that of 3.72 mg/g in Keemun dark tea. High residue of catechins may contribute to the strong taste of Keemun dark tea.

Keemun *Maofeng* green tea processed from *Zhuye* breed had its typical aroma composition compared with Huangshan *Maofeng* green tea or other green tea in China. The special aroma component 2,4-dimethyl -benzaldehyde was detected rich in Keemun green tea with a ratio of 32.22% in the total essential oils, while it was not found in Xinyang *Maojian* (Xinyang, Henan province), Huangshan *Maofeng* （Huangshan, Anhui province）and Xihu *longjing* (Hangzhou，Zhejiang province) (Liu et al., 2016). Dimethyl sulfide, with a special delicate fragrance of fresh green tea, was detected in Keemun *Maofeng*, while was also not found in the other green tea mentioned above (Liu et al., 2016). But the difference of the ratio of aldehyde or alcohol in the essential oils between the 3 kinds of tea was not so much as expected (table 10).

Table 10. Ratio of different aroma component category in the 3 samples (%)

	aldehyde	alcohol	ketone	alkene	alkene	acid	heterocycles
black tea	45.05	39.33	2.95	1.16	1.16	4.83	0.600
dark tea	39.79	26.59	2.25	0.95	0.95	8.46	11.9
green tea	50.39	31.99	0.44	0.12	0.12	6.66	0.447

2-ethylhexyl salicylate, methyl salicylate, homosalate were found in all of the 3 kinds of Keemun tea (Table 5) and these were believed to belong to the chestnut-fragrance components (Yin et al., 2018**).**

Linalool oxide and geraniol in the spiral-shaped Keemun black tea was much higher than that in traditional Keemun black tea (He et al., 2015, Mao et al., 2018, Xiao et al., 2017). The combination of roasting drying with oven drying technique in the new processing method may be considered benefited to the fragrance development for black tea, and would be recommend as a normal procedure for black tea processing.

Among the other aroma constituents, phenylethyl alcohol, 2,4-dimethyl-benzaldehyde, 6-methyl-5-Hepten-2-one, 2-ethyl-1-hexanol, and 2-methyl-butanal, which represent the sweet flavor or fragrance of flowers (Xiao et al., 2017) were also enriched in the spiral-shaped black tea (table 5)**.**

Heterocycles and acid indeed showed a high ratio in Keemun dark tea aroma (table 10). However, the stale taste chemicals 3,5,5-trimethyl-2-cyclohexene-1 - ketone, 5- amino -1- ethyl pyrazole, 1,2,3- trimethoxy benzene which exist in Puer tea, Fuzhuan tea (Xu et al., 2007) were not found in Keemun dark tea (table 5). 15 Fuzhuan samples were analyzed and found that acid was 18.85% of the total essential oils (Xu et al., 2007), that was much higher than that in Keemun dark tea (table 10).

The long time non-microorganism storing for dry primary tea, the dewing processing, the cotton covered final drying processing were all unique for Keemun dark tea producing, and may help to form its special quality, this leaves to be further investigated.

5.2 Anti-Oxidation checking

The most important value of tea as a drink is its health function, and it is widely accepted that radical scavenging ability represents one of the most important quality factors for tea products (Brand-Williams et al., 1995, Lee et al., 2016). A great number of studies have found that the antioxidant properties, antibacterial, antitoxin, anti-mutagens and anti-inflammation of tea are mainly attributed to polyphenolic compounds and the pigments formed by the polymerization of polyphenols including the flavanols (Wolfe & Liu, 2007; Zhang et al., 2013).

High content of flavanols in green tea, pigments in black tea and dark tea are the main contribute to the anti-oxidation function of tea. In the present study, in all of the 3 kinds of teas investigated, for DPPH radical and superoxide anionic radicals scavenging, ethanol tea extraction was more efficient than the water tea extraction (Tables 6, &7). It can be inferred that the ester solution components in green tea like insoluble amylose, condensed tannin and the pigments like TFs, TRs and some non-identified components in black tea and dark tea have also a strong ability for radical scavenging (Tan et al., 2012, Mahejabeen et al., 2015).

However, for hydroxy radical scavenging rate, only in the case of black tea, the ethanol extraction was better than the water extraction, whereas in the cases of dark tea and green tea, hydroxy radical scavenging rate is in contrary that water tea extraction was better than the ethanol extraction (Table 8).

On the other hand, there was no a common trend for the impact of extracting methods for different kinds of tea on different radicals scavenging. 3 kinds of tea had a similar DPPH radical scavenging rate (Table 6), whatever the extracting way. Yet, for superoxide anionic radicals reaction system, both in the situation

of water extracts and ethanol extracts, the scavenging rate is dark tea > green tea >black tea (Table 7). Judged only by free radical scavenging efficiency for health function of all kinds of tea, dark tea seemed to be better than green tea in this study.

For general reducing power, green tea was the strongest, followed by black tea and dark tea in sequence, this was consistent with the content of catechins in the 3 kinds of tea which was regard as highly effective radicals scavenging index. However it was also clear that the general reducing power of tea can not always reflect their radical scavenging efficiency, for catechins may not be the only components capable of scavenging free radicals (Wolfe & Liu, 2007, Sharma & Rao, 2009). Which components are more important for radicals scavenging besides catechins are left for further exploring.

Usually green tea gets more attention for its radicals scavenging ability and reducing power. However, more and more evidence supports the point that black tea and dark tea may have their advantages for health function with their complicated pigments or even aromas (Higdon & Frei, 2003; Fu et al., 2011). In fact, it has been confirmed that black tea can protect the gastrointestinal mucosa by preventing oxidation of tissues, repairing oxidative damages and interacting with gastric mucus, glutathione and other substances (Maity et al., 2003, Mahejabeen et al., 2015).

6. CONCLUSION

Keemun gets noted by the world for producing the most quality Congou black tea, to explore the processing potential of the traditional fine breed Keemun *Zhuye* cultivar, a spiral- shaped black tea, a mellow taste dark tea（*Ancha*） and a vivid green color *Maofen* tea was created. Though there was also a post -ferment produce schedule in the processing of *Ancha* dark tea, no tare taste or aging aroma was noted in it. Chemicals checking showed EGC, GCG has a higher content in dark tea than that in the green tea while there were less acids composition existed in the dark tea, that may be attributed to the strong and mellow taste of the *Ancha* dark tea.

Linalool oxide and geraniol in the spiral-shaped Keemun black tea was much higher than that in traditional Keemun black tea, phenylethyl alcohol, 2,4-dimethyl-benzaldehyde, 6-methyl-5-Hepten-2-one, 2-ethyl-1-hexanol, and 2-methyl-butanal (represent the sweet flavor or fragrance of flowers) were also enriched in the spiral-shaped black tea. The combination of roasting drying with oven drying technique may benefit much to the fragrance development of black tea, and would be recommend as a normal procedure for black tea processing.

The special aroma component 2,4-dimethyl-benzaldehyde and dimethyl sulfide (represent the fresh flavor) was rich in Keemun green tea implied that both of the quality black tea and green tea could be derived from the special breed Keemun *Zhuye* cultivar.

Though green tea got the most high general reducing power, *Ancha* dark tea has a more effect for superoxide anionic radicals and DPPH radical scavenging ability than that of green tea and black tea. This suggests that dark tea in some aspects has its advantages for heathy promoting.

Our work may gave a further suggestion for the possible innovation way of traditional Congou black tea and dark tea processing technique and the mutipurpose utilization of traditional find breed of tea plant.

Abbreviation

RH, relative humidity

Vc，vitamin C，ascorbic acid

GA, gallic acid

GC, gallocatechin

EGC, epigallocatechin

C，catechin

EC，epicatechin

EGCG, epigallocatechin gallate

GCG, gallocatechin gallate

CG，catechin gallate

Declarations

Supplementary data to this article can be found online at FPPN..

Competing interests: There is no any competing interests on this paper.

Funding: This study was supported by Jiangsu Agricultural Science and Technology Independent Innovation Fund (CX(18)3034), Jiangsu Key Research & Development Plan for Morden agriculture (BE2019320). The purpose of the fund of CX(18) 3034 is improving the quality of blacktea and developing new products in Jiangsu province, obviously, as one of the most famous tea, Keemun blacktea's quality, proceesing and its function has a great impact on the innovation of tea industry in Jiangsu province. Fund of 2016YFD0200905 aims at the reducing of agicultural imputs in tea garden, Jiangsu tea product's area and Anhui tea product's area (including Keemun county) was list in the demonstration areas, how the agicultural imputs decreasing will affect the tea quality is one of the key problems need to be considered.

Authors' contributions: Mr Ronglin Li conceived and conducted the experiments, and wrote the paper. Mr Zhenming Hu, Miss Zeyi Ai help to conceive and design the experiments, reviewed the text. Mr Jianhua Yang and Hao Zheng partly conducted the experiments. Mr. Zhengtao Chen and Mr. Haihua Shi helped in preparing the samples.

Acknowledgements: Dr. Jianliang Lu in Zhejiang University gave help in analysis of the aroma composition. Dr. Lisheng Qian in Zhejiang University read the script and gave valuable advice for grammar and structure of the paper.

Authors' information: Mr Ronglin Li, professor in Jiangsu academy of agricultural sciences, research interesting: tea science and technology, food chemistry.

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