

Agronomic Traits and Quality Screening of Superior *Camellia oleifera* in Tropical Areas

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Abstract In order to breed high-yield and high-quality *Camellia oleifera* varieties, promote the quality upgrade of Hainan camellia tea oil. In this study, through the investigation and analysis of different production areas of *Camellia oleifera* in Hainan, excellent single plants were selected from the main areas of *Camellia oleifera* planted in Hainan, focusing on the main botanical traits, main fruit traits, and differences in economic traits, tea oil fatty acid composition and content, the correlation between fatty acids and other aspects are analyzed specifically for the agronomic traits and tea oil quality of excellent individual plants. The results showed that the botanical characteristics are as follows: tree height is between 1.4 m and 8.4 m, crown width is between 1.1×1.0 and 8.0×7.3 m, the initial flowering period is generally from late October to early November, and the flowers are all white. The fruit maturity period is also between mid-October to early November, etc.; the fruit traits show that the horizontal diameter of the fruit is 38.67 ~ 55.32 mm, the longitudinal diameter of the fruit is 27.64 ~ 40.71 mm, and the fruit shape is peach-shaped or orange-shaped. Green or blue-brown, etc.; all indicators of economic traits are significantly different ($p < 0.01$), mainly due to the differences in the agronomic traits of the excellent trees, resulting in different degrees of the economic traits of the fruits, and also affecting the yield and the quality of the excellent trees. The study found that the superior tree traits in Wuzhishan area are superior to other areas, and the oleic acid content and quality of tea oil in Ding'an Shitang area are higher than other areas. The selected excellent single plant has better agronomic traits, high fruit yield, high oil content and strong disease resistance. This study specifically analyzed and evaluated the excellent individual plants in different producing areas of *Camellia oleifera* in Hainan, and revealed the differences in agronomic traits and tea oil quality in different areas of *Camellia oleifera*, and provided a reference for the breeding work of *Camellia oleifera* in Hainan.

Keywords *Camellia* breeding; Superior tree; Agronomic character

Camellia oleifera and *Canarium album*, *Elaeis guineensis*, *Cocos nucifera* are known as Four Woody Oil Plants in the World. *Camellia* oil is rich in nutrients and has the reputation of "East Olive Oil" (Chen et al., 2005). *Camellia oleifera* has a long history in China, mainly distributed in southern provinces of China such as Guangxi and Yunnan. Yuan et al. (2014) reported that the Hainan *Camellia oleifera* has rich genetic characteristics for the unique geographical location, agronomic traits and tea oil quality are different from those in the inland. In Hainan, *Camellia oleifera* industry is also an ecological characteristic industry with broad development and application prospects. Fully exploring the characteristic resources of Hainan *Camellia oleifera* and cultivating excellent new varieties are of great and far-reaching significance for accelerating the structural reform of Hainan's agricultural supply side, winning the battle against poverty and promoting farmers' income and prosperity. However, the development and research of *Camellia oleifera* in Hainan started late, so these problems such as low yield per plant, low oil content and low efficiency have become the key problems restricting the development of *Camellia oleifera* in Hainan.

At present, the main producing areas of *Camellia oleifera* in Hainan Province have small planting area, low yield and low oil content, and the quality of camellia oil needs to be improved. In order to screen out individual *Camellia oleifera* with good camellia oil quality, promote quality upgrade, and improve the economic benefits and

market competitiveness of Hainan *Camellia oleifera* seed oil, this study analyzed the main characteristics, oil content, fatty acid composition and content of samples collected from different main production areas, and screened out excellent individual plants, which provided a reference for improving the quality of Hainan *Camellia oleifera* seed oil.

1 Results and Analysis

1.1 Excellent single major plant traits

The botanical characters of *Camellia oleifera* from different producing areas are different, which is closely related to the local varieties and growth environment. The tree age of the investigated individual is about 10~50 years; The 'WZSTZ-3' (Tengzhai Village, Wuzhishan City) with the highest tree height of 8.4 m, and the 'QHX-23' (Qionghai City) with the smallest tree height of 1.4 m, with an average of 4.7 years; The crown width is between 1.1×1.0 and 8.0×7.3 m, of which 'WZSTZ-3' (Tengzhai Village, Wuzhishan City) has the largest crown width. The larger crown width and the larger leaf photosynthesis area, which directly affects the yield and quality of tea oil. According to comprehensive research and judgment, its botanical characters are as follows: Wuzhishan Tengzhai>Ding'an Shitang>Wuzhishan Baoguo, Shiha>Chengmai>Danzhou>Qionghai. Therefore, in the work of *Camellia oleifera* breeding, this study will focus on the excellent individual plant in Wuzhishan region. Among the investigated individual plants, the initial flowering period is generally from late October to early November, the flower color is white, and the fruit maturity period is also between mid-October and early November. The anthrax rate is less than 3%, which creates good conditions for obtaining high yield and ensuring quality (Table 1; Figure 1).

Table1 Excellent single major plant traits

Variety number	Tree age (year)	Height (m)	Crown width (m×m)	At the beginning of flowering	Color	Fruit ripening stage	Anthrax rate (%)
DAST-2	40	6.5	5.0×4.2	Late October	White	Early October	<3
DAST-4	50	5.7	7.8×5.8	Early November	White	Early November	<3
DAST-5	30	5.3	2.5×3.2	Late November	White	Mid-October	<3
QHX-23	10	1.4	1.1×1.0	Late October	White	Mid-October	<3
QHX-24	10	2.2	2.4×2.5	Late October	White	Mid-October	<3
QHX-25	10	1.5	2.4×2.6	Late November	White	Mid-October	<3
QHX-29	10	2.1	1.8×1.7	Late November	White	Mid-October	<3
CMF-1	30	4.1	5×4.1	Early December	White	Mid-October	<3
CMF-2	40	4.5	5×4.5	Late November	White	Mid-October	<3
CMF-4	40	4.3	3.8×3.4	Late October	White	Mid-October	<3
CMF-6	20	4.2	4.1×3.1	Late October	White	Mid-October	<3
WZSTZ-1	22	6.2	7.9×6.8	Late November	White	Mid-October	<3
WZSTZ-2	20	7.5	8.0×7.1	Late October	White	Mid-October	<3
WZSTZ-3	30	8.4	8.0×7.3	Early November	White	Mid-October	<3
WZSTZ-4	26	6.2	5.2×3.3	Early November	White	Mid-October	<3
WZS-1	36	4.5	5.1×4.8	Late October	White	Mid-October	<3
WZS-2	40	4.1	4.9×4.6	Late October	White	Mid-October	<3
DZ-1	20	6.1	4.3×3.6	Early November	White	Mid-October	<3

1.2 Fruit characteristics of excellent single plant

There were significant differences in individual fruit characters from different producing areas ($p<0.01$). Through the processing, analysis and screening of a series of data in R language, the main fruit characters were as follows: Wuzhishan Tengzhai>Chengmai> Wuzhishan Baoguo, Shiha> Danzhou> Qionghai>Ding'an Shitang. 'WZSTZ-1' (Tengzhai Village, Wuzhishan City) with the largest horizontal diameter of 55.32 mm, while the smallest is 'QHX-29'(Qionghai) of 38.67 mm. The variation range of fruit longitudinal diameter is 27.64~40.71 mm, with the average value of 34.54 mm, among which the longitudinal diameter of 'CMF-1' (Chengmai County) is the

largest. The pericarp thickness ranges from 1.12 to 4.58 mm, with an average of 2.87 mm, of which 'WZSTZ-2' (Tengzhai Village, Wuzhishan City) has the largest pericarp thickness. Generally, fruit characters directly affect its yield and quality. The fruit shape of the investigated varieties is peach-shaped or orange-shaped, and the fruit color is cyan or cyan brown, and a few are cyan red. The areola is top level and concave convex. The fruit edge and ditch are characterized by no edge and gap, and the number is different (Table 2).



Figure 1 Selected outstanding strains investigated

Note: A: The region of Wuzhishantenzhai; B: The region of Wuzhishanzhaha

Table 2 Fruit characteristics of excellent single plant

Variety number	Fruit diameter (mm)	Fruit longitudinal diameter (mm)	Pericarp thickness (mm)	Fruit shape	Fruit color	Areola	Edge	Ditch
DAST-2	52.67	30.28	1.13	Peach shape	Cyan	Top level	No edge	No gap
DAST-4	51.22	27.64	1.16	Peach shape	Cyan	Top level	No edge	No gap
DAST-5	48.78	28.89	1.18	Peach shape	Cyan	Top level	No edge	No gap
QHX-23	39.67	30.12	2.31	Orange shape	Green brown	Top concave	Three edges	Three gaps
QHX-24	40.16	31.18	2.23	Orange shape	Green brown	Top concave	No edge	No gap
QHX-25	41.23	30.56	2.70	Peach shape	Green brown	Top concave	Three edges	Three gaps
QHX-29	38.67	29.16	2.13	Orange shape	Cyan	Top concave	No edge	Three gaps
CMF-1	45.67	40.71	3.12	Orange shape	Red brown	Top level	Three edges	No gap
CMF-2	51.72	37.59	2.14	Orange shape	Cyan	Top concave	Four edges	No gap
CMF-4	42.06	35.71	3.16	Peach shape	Green brown	Top concave	No edges	Four gaps
CMF-6	44.92	34.94	2.11	Orange shape	Cyan	Top level	Two edges	Three gaps
WZSTZ-1	55.32	36.23	3.13	Orange shape	Cyan	Top concave	No edge	No gap
WZSTZ-2	49.14	36.23	4.58	Orange shape	Cyan	Top concave	No edge	No gap
WZSTZ-3	41.12	38.34	3.76	Orange shape	Cyan	Top concave	No edge	No gap
WZSTZ-4	52.13	39.87	2.39	Orange shape	Cyan	Top concave	No edges	No gap
WZS-1	42.67	39.29	1.12	Peach shape	Green brown	Top concave	No edge	No gap
WZS-2	41.45	38.21	1.46	Peach shape	Green brown	Top level	No edge	No gap
DZ-1	41.68	36.78	1.36	Peach shape	Green brown	Top level	No edge	No gap

1.3 Analysis of differences in economic traits of excellent trees

There were significant differences in fruit economic characters (fresh fruit quality, seed weight, single grain weight, seed ratio of fresh fruit, seed oil yield, fresh fruit oil rate, unit crown yield and oil production) in different producing areas of *Camellia oleifera* in Hainan ($p < 0.01$). The different producing areas and excellent trees investigated in this experiment are representative, which represent the main producing areas and main planting varieties of *Camellia oleifera* in Hainan. The difference of economic characters is significant, which is mainly due to the difference of agronomic characters of excellent trees, resulting in different degrees of difference of economic characters in fruits, and also affecting the yield and quality of *Camellia oleifera* excellent trees. The differences of economic characters may also be related to the local varieties, growth environment, planting methods and other factors (Table 3).

Table 3 Analysis of differences in economic traits of excellent trees

Project	Fresh fruit quality (g)	Seed weight (g)	Single grain weight (g)	Seed ratio of fresh fruit (%)	Seed oil yield (%)	Fresh fruit oil rate (%)	Unit yield (kg/m ²)	crown Oil Production (kg/m ²)
F value	44.6	199	3.78	70.92	174.1	6.98	39.69	29.93
p value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

1.4 Composition and content of fatty acid of tea oil in different producing area

The fatty acids of *Camellia oleifera* seed oil from different producing areas are different. The main components of tea oil are oleic acid, linoleic acid, linolenic acid, stearic acid and palmitic acid, accounting for more than 98% of the total fatty acids. The oleic acid content of monounsaturated fatty acid is 70.71%~86.21%, with the average value of 80.82%, and the coefficient of variation is 4.46%, indicating that the content of oleic acid is relatively stable. Comparative analysis showed that the proportion of oleic acid was as follows: Ding'an Shitang>Wuzhishan>Qionghai>Chengmai>Danzhou. The content of linoleic acid is 2.83%~13.22%, the average value is 6.52%, and the coefficient of variation is 37.61%, indicating that the content of linoleic acid in tea oil from different production areas changed significantly. The stearic acid content is 1.69%~3.43%, with the average value of 2.56%, and the coefficient of variation is 21.24%, indicating that the stearic acid content of tea oil in different production areas is different. The content of palmitic acid is 7.51%~12.92%, with an average of 9.04% and a coefficient of variation of 15.23%, indicating that the content of palmitic acid in tea oil from different producing areas is stable. In a word, there are certain differences in fatty acids of tea oils from different producing areas in Hainan. The oleic acid content is relatively stable, while the linoleic acid content varies greatly (Figure 2).

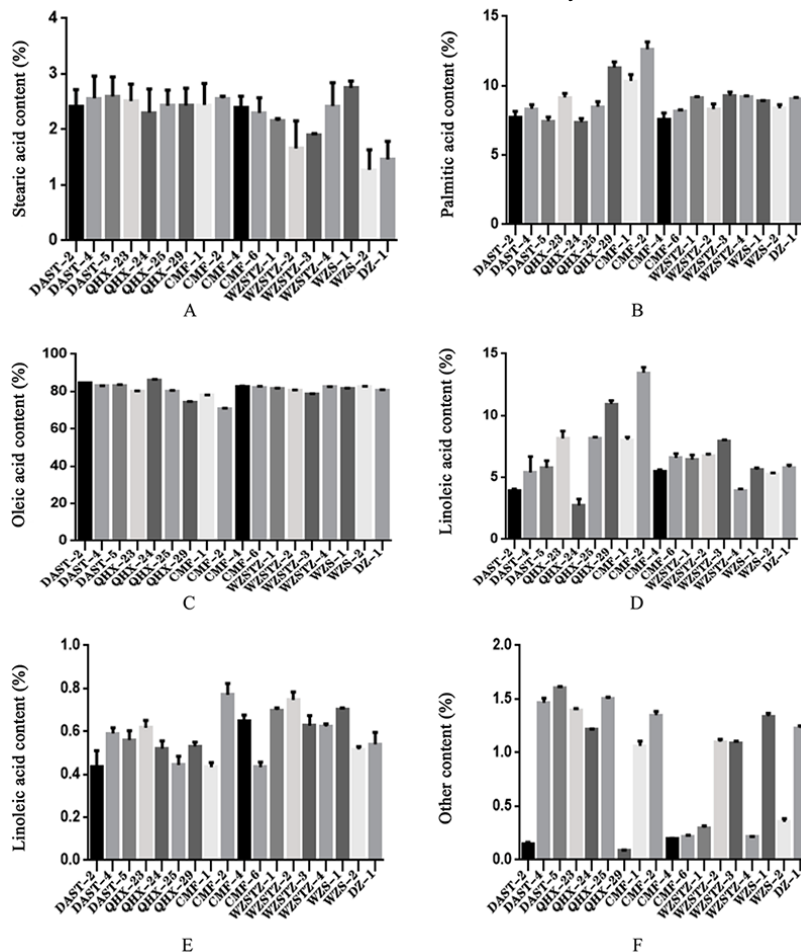


Figure 2 Composition and content of fatty acid in tea oil

Note: A: Content of stearic acid; B: Palmitic acid content; C: Oleic acid content; D: Content of linoleic acid; E: Content of linoleic acid; F: Other content

1.5 Correlation between four major fatty acids

The total content of oleic acid, linoleic acid, palmitic acid and stearic acid account for more than 98% of fatty acids (Meng et al., 2017). The content of monounsaturated fatty acids (oleic acid) in the investigated areas is relatively stable, all around 80%, and the content of linoleic acid is significantly different. Oleic acid has a very significant negative correlation with the content of linoleic acid and palmitic acid, while the content of linoleic acid is relatively unstable, with a large degree of variation, and is gradually transformed into oleic acid (Figure 3).

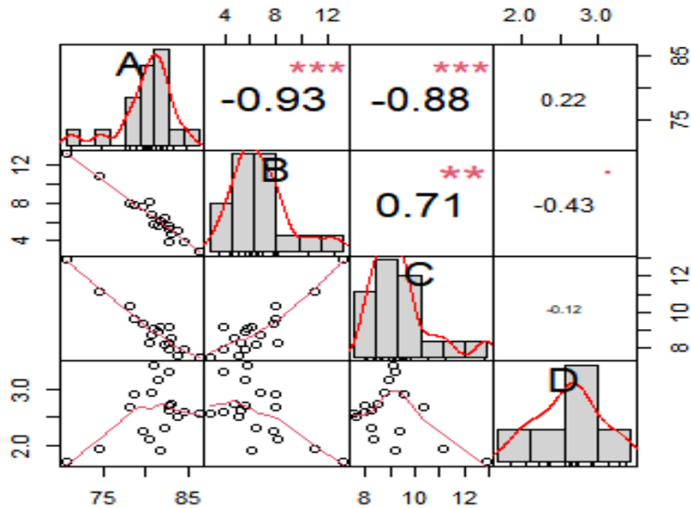


Figure 3 Correlation between fatty acids in tea oil

Note: A: Oleic acid; B: Linoleic acid; C: Palmitic acid; D: Stearic acid; **: Significant correlation at the 0.01 level; *: Significant correlation at the 0.05 level

2 Discussion

At present, Hainan *Camellia oleifera* breeding faces the problems of low yield and poor quality. One of the effective methods to improve varieties is to cultivate excellent varieties based on excellent individual plants. In the research of *Camellia oleifera* breeding in Hainan, there have been much previous research in a certain area, but the breeding value and economic benefit are not significant, and there are some limitations (Feng et al., 2017). Comprehensive analysis of agronomic traits and tea oil quality of superior trees in different production areas in Hainan can effectively solve the problems of yield and quality (Xu, 2018). Combined with the advantages of superior tree traits and tea oil quality in different production areas, a new breeding objective of high yield and high quality is put forward. All indicators of economic traits were significantly different ($p < 0.01$). Wang et al. (2016) reported that the differences of economic traits were mainly caused by climate, variety differences and other factors. The results of this study showed that the main reason was due to the differences of growth environment and agronomic traits in different producing areas, and may be affected by seed selection, climate conditions, planting technology and other factors. Yuan et al. (2012) reported that Hainan *Camellia oleifera* seed oil is rich in content and its quality is higher than that in the inland. The four main fatty acids of tea oil, oleic acid, linoleic acid, palmitic acid and stearic acid, are closely related. In this study, the agronomic traits of *Camellia oleifera* superior trees in Wuzhishan area are better than those in other areas. The content of oleic acid in tea oil in Ding'an area is relatively stable and rich, which has obvious advantages over other areas. Therefore, to create conditions for breeding high-yield and high-quality *Camellia oleifera* varieties, we will focus on the above two areas in Hainan *Camellia oleifera* breeding.

Camellia oleifera varieties are relatively less affected by environmental factors, but agronomic characters are more intuitive as external characteristics, and affect their yield and quality. How to maintain good agronomic characters and improve the quality of tea oil is an essential link in Hainan *Camellia oleifera* breeding, and it is also the direction of further exploration on the basis of inheriting previous studies in the future. In this study, the 'DAST-2' sample with the highest oil content (55.32%) was collected in Ding'an County, Hainan Province. The tree age is about 40 years, indicating that Ding'an area is the main production area with better tea oil quality. In the

future, we will focus on the detailed and in-depth research on the superior trees in this area in the breeding process. The relative content of monounsaturated fatty acids (oleic acid) in tea oil is 70.71%~86.21%, with an average of 80.82%. The relative content of unsaturated fatty acids is higher than that of olive oil published by WHO (Tang et al., 2013; Zhu et al., 2017). Compared with the inland, its content and quality have significant advantages. Accelerating the cultivation of new varieties with high yield and quality is of great significance to improve the economic benefits and competitiveness of Hainan *Camellia oleifera*.

Through a series of experimental analysis, it is proved that agronomic characters affect the yield and quality of *Camellia oleifera*. And its rich variation characteristics provides a large number of breeding materials for *Camellia oleifera* breeding in Hainan.

3 Materials and Methods

3.1 Experimental materials

This study investigated Ding'an, Chengmai, Qionghai, Wuzhishan and Danzhou in Hainan from June 2018 to November 2019. According to the breeding requirements of high yield and high quality (it mainly includes the arborization of trees, long lanceolate leaves, orange-shaped fruits, high yield, 19.67%~25.32% of seed oil yield and 2.32 kg of crude oil per plant. The main phenological phase: November to February of the previous year is the flowering period, March to April is the fruit setting period, May to August is the rapid fruit expansion period, and September to November is the fruit ripening period and flower bud differentiation period. The oleic acid content of tea seed oil is 70.18%~80.625%, linoleic acid is 3.11%~5.84%, saponification value is 292.54 mg/g, iodine value is 75.73 g/100 g, and acid value is 0.53, peroxide value is 1.99%. In general, tea seed oil has strong storage resistance, oxidation resistance and short fatty acid carbon chain), 18 core individual plants were selected, and the superior trees of *Camellia oleifera* Abel were located (Table 4).

Table 4 Information on *Camellia oleifera* samples surveyed

Variety number	Sample name	East longitude	North latitude	Location of collection
DAST-2	Dingan shitang 2	110°13'39.95"E	19°24'32.13"N	Ding 'an County, Hainan Province
DAST-4	Dingan shitang 4	110°13'39.75"E	19°24'31.70"N	Ding 'an County, Hainan Province
DAST-5	Dingan shitang 5	110°13'40.65"E	19°24'30.56"N	Ding 'an County, Hainan Province
QHX-23	Qionghai xuan 23	110°30'21.80"E	19°5'9.93"N	Qionghai City, Hainan Province
QHX-24	Qionghaixuan 24	110°30'22.86"E	19°5'9.83"N	Qionghai City, Hainan Province
QHX-25	Qionghaixuan 25	110°30'23.75"E	19°5'9.74"N	Qionghai City, Hainan Province
QHX-29	Qionghai xuan 29	110°30'21.84"E	19°5'10.02"N	Qionghai City, Hainan Province
CMF-1	Chengmai fu 1	109°30'37.95"E	18°51'38.97"N	Chengmai County, Hainan Province
CMF-2	Chengmaifu 2	109°30'38.05"E	18°51'38.95"N	Chengmai County, Hainan Province
CMF-4	Chengmaifu 4	109°30'39.95"E	18°51'38.92"N	Chengmai County, Hainan Province
CMF-6	Chengmai fu 6	109°30'39.95"E	18°51'38.84"N	Chengmai County, Hainan Province
WZSTZ-1	Wuzhishantenzhai 1	109°57'26.10"E	19°24'11.47"N	Tenzhai Village, Wuzhishan City, Hainan Province
WZSTZ-2	Wuzhishantenzhai 2	109°57'26.47"E	19°20'11.52"N	Tenzhai Village, Wuzhishan City, Hainan Province
WZSTZ-3	Wuzhishantenzhai 3	109°57'26.36"E	19°20'10.96"N	Tenzhai Village, Wuzhishan City, Hainan Province
WZSTZ-4	Wuzhishantenzhai 4	109°57'26.80"E	19°20'10.53"N	Tenzhai Village, Wuzhishan City, Hainan Province
WZS-1	Wuzhishant1	109°27'55.82"E	18°40'46.33"N	Zhaha Village, Wuzhishan City, Hainan Province
WZS-2	Wuzhishan2	109°26'38"E	18°41'59"N	Baoguo Village, Wuzhishan City, Hainan Province
DZ-1	Danzhou1	109°23'45.84"E	19°20'21.84"N	Danzhou City, Hainan Province

3.2 Observation of agronomic characters

On the basis of consulting relevant literature (Zheng et al., 2016), the research group visited local farmers and Agricultural and Forestry Departments to conduct a comprehensive field investigation on the distribution of *Camellia oleifera* resources in different producing areas of Hainan. At the same time, the *Camellia oleifera* forests in the main production areas for more than 10 years were investigated (Fu et al., 2014, Chinese Journal of Tropical

Agriculture, 34(6): 41-43). We mainly visited and investigated Ding'an, Qionghai, Chengmai, Danzhou and Wuzhishan in Hainan Province. These cities and counties basically cover the main producing areas of *Camellia oleifera* in Hainan Province. Excellent individual plants were selected from different producing areas of *Camellia oleifera* in Hainan Province, and their agronomic characters such as stem circumference, plant height, crown width, leaf length, leaf width, fruit longitudinal diameter and fruit horizontal diameter were measured and analyzed.

3.3 Oil content measurement

The *Camellia oleifera* seeds are shelled, peeled and ground. 5 g samples are weighed, wrapped with degreasing filter paper and tied with rope to ensure no residue leakage in the extraction process. Then they are placed in Soxhlet extractor and extracted by reflux with petroleum ether with boiling range of 60°C~90°C for about 6 h. Until the petroleum ether no longer appears yellow, then continue to evaporate, recover the extraction solvent petroleum ether, and finally get tea oil. Put it in a small test tube and store it in the refrigerator at 4°C. After the temperature of the Soxhlet tube drops, take out the filter paper package, pay attention to avoid direct touch by hand, put it into the oven, dry it at a constant temperature for 1 h, take it out and put it in the dryer until all the petroleum ether volatilizes, and weigh the remaining extract when it is completely dry. The oil content is the difference between the front and rear mass divided by the original weight. Repeat the extraction for three times and take the average value.

3.4 Methyl esterification of *Camellia oleifera* seed oil

Take 400 µL sample and put into 10 mL test tube and add 200 µL KOH-CHH (0.5 mol/L) and H₂SO₄-methanol solution, shake fully, stand for 30 min, add a little distilled water, take the supernatant after stratification for analysis (Zhong et al., 2015), and determine the fatty acid composition by Shimadzu GC-2014 Gas Chromatograph in Japan.

3.5 Analysis of fatty acids in *Camellia oleifera* seed oil

The contents of fatty acids oleic acid, linoleic acid, linolenic acid, palmitic acid and stearic acid in tea oil were measured by gas chromatography. The relative contents of each fatty acid were calculated by normalization method according to the peak area. The requirements of gas chromatograph are as follows: injection volume 1 µL; The temperature at the column is 220°C, and the pressure is 13.4 pa; Column model is Agilent122-103. Temperature rise procedure is as follows: the initial temperature is 100°C, keep it for 1 min, and then raise the temperature to 240°C at the rate of 6°C/min for 12 min. The detector temperature is 270°C, the air flow is 45 L/min, the hydrogen flow is 4 L/min, and the tail gas flow is 4 L/min (Aranda et al, 2004; Li et al., 2012).

3.6 Data analysis

Excel 2007 software was used for data processing, and SPSS19.0 software was used for difference significance analysis. R4.0 language software was used for correlation analysis of main fatty acids.

Authors' Contributions

WH and DYJ are the executors of the experimental design and research of this study. WH, LYH and SJM completed data analysis and the writing of the first draft of the paper. ZY participated in the experimental design and analysis of the experimental results. LHG is the designer and person in charge of the project, guiding experimental design, data analysis, paper writing and revision. All authors read and approved the final manuscript.

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