

Fruit annual changes in morphological characteristic and essential oil accumulation of *Litsea cubeba*

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Abstract

Litsea cubeba (Lour.) Pers. is an important spice plant in southern China. The whole plant of *Litsea cubeba* contains essential oils, among which the fruit has the highest essential oil content. And there is a significant market demand and widespread use of essential oil of *Litsea cubeba* fruit. However, there are few systematic studies on the growth and development of the *Litsea cubeba* fruit. This study aims to determine the regularity of annual changes in fruit morphology, essential oil content, and components of *Litsea cubeba* essential oil to determine the optimal harvest period and improve the utilization efficiency of *Litsea cubeba* resource. The results show that the annual change of fruit morphology of *Litsea cubeba* was consistent with the double "S" curve, which shows a general trend of rapid growth first, then slow growth, and finally rapid growth again. While the annual change of the essential oil of *Litsea cubeba* fruits was consistent with a single "S" curve, which shows a general trend of slow growth first, then rapid growth, and finally decreasing rapidly. Correlation analysis showed a significant negative correlation between morphological changes and essential oil accumulation in *Litsea cubeba* fruit. Combining the growing periods of fruit morphology and essential oil accumulation, the growth and development of *Litsea cubeba* fruit can be divided into four general periods: the first rapid growth period (rapid growth in fruit diameter and fruit weight with slow accumulation of essential oil), the essential oil accumulation period (rapid accumulation of essential oil with slow growth in fruit diameter and fruit weight), the second rapid growth period (fruit diameter and fruit weight increased rapidly for the second time while the essential oil content started to decrease), and the shriveling period (fruit diameter and weight and essential oil content all decreased rapidly). This study further confirmed that citral was the highest component in the essential oil of *Litsea cubeba*. When citral extraction is needed, the fruit can be harvested 139–149 days after anthesis.

1. Introduction

Litsea cubeba (Lour.) Pers. is a deciduous shrub or dungarunga of the LitseaLam in the family Lauraceae. As a dioecious plant, it usually produces flower buds in the winter and flowers in the following spring. *Litsea cubeba* fruit is a berrylike spherical drupe, which is green when immature and black when fully ripening (Chen et al., 2013). *L. cubeba* is a light-loving plant with a shallow root system. It tends to grow in barren hills or wasteland at an altitude of 300 to 1800 m, as well as in shrubby areas and sparse woods. In China, there are 46 known species of *Litsea cubeba*. Nearly 80% of the *Litsea cubeba* resource is wild and only a few plantations exist (Chen et al., 2013). *Litsea cubeba* is an important source of spices and essential oils (Li et al., 2019). Besides, *Litsea cubeba* has long been used as a folk medicine in the Dai minority of China, which was recorded as early as 2,500 years ago in the Book of Shellfish Leaves (Lin et al., 2013). *Litsea cubeba* oil is often used as a flavor enhancer in cosmetics, food, and cigarettes (Li et al., 2014). Due to its antioxidant, anti-inflammatory, genotoxicity, antibacterial activity and other functional properties, it is now commonly used to make drugs, bacteriostatic agents, food additives and insecticides (Li et al., 2014; Wang et al., 2010; Liu et al., 2012; Gogoi et al., 2018).

As a woody spice plant with high application value, *Litsea cubeba* has become a research hotspot. The hotspot mainly focuses on the seedling propagation and cultivation technology of *Litsea cubeba* (Wei et al., 2017), the extraction, processing and application of *Litsea cubeba* essential oil, and the application of *Litsea*

cubeba in medicine. Studies on the essential oil of *Litsea cubeba*, including optimization of the extraction process of *Litsea cubeba* essential oil (Huang et al., 2021), analysis of essential oil components from different parts of *Litsea cubeba* (Ho et al., 2021; Wang et al., 2010), and rich biological characteristics of *Litsea cubeba* essential oil such as antioxidant activity (Jae-Kwan et al., 2005) and antibacterial or antifungal activity (Hu et al., 2019; Wu et al., 2019). There are also some studies on the application of *Litsea cubeba* essential oil, including the potential application of *Litsea cubeba* essential oil in the cosmetic industry (Huang et al., 2013) and the possibility of *Litsea cubeba* essential oil as an efficient and environmentally friendly antifungal fumigant (Li et al., 2016). In addition, the medical application of *Litsea cubeba* has been extensively studied in recent years. Animal experiments conducted by Lin et al. (2013) suggested that *Litsea cubeba* could be a potential drug for treating human arthritis. Kuan-Tseng et al. (2021) found that *Litsea cubeba* fruit powder has a good potential to develop functional drugs against Alzheimer's disease. Furthermore, *Litsea cubeba* nucleolus oil has also been found to be exchanged for biodiesel production (Li et al., 2021).

Fruit development and ripening are important stages in higher plants' life cycle. As the most widely used plant parts, fruit development will affect most plant species' quality and economic value. Known research results show that external factors such as mean annual precipitation, mean annual temperature, temperature difference and light have obvious effects on fruit yield, cell size, cell number, and intrinsic fruit components. For example, the correlation analysis conducted by Yuan et al. (2021) showed that the mean annual precipitation and mean annual temperature were positively and significantly correlated with the yield per plant of *Litsea cubeba*. Osatuke et al. (2021) found that the sugar and acidity of strawberries were positively correlated with the temperature difference during fruit ripening. The study by Okello et al. (2015) showed that light strongly affected the increase in cell number and the decrease in cell size in tomatoes. In addition, the intrinsic substances of fruit cells such as ethylene (Chervin et al., 2004; Martínez et al., 2013), organic acids (Batista-Silva et al., 2018), and mitochondrial ascorbic acid (Alhagdow et al., 2007), as well as the physiological response of the cell (Bertin et al., 2002) can have an important impact on fruit development.

In order to use *Litsea cubeba* scientifically and efficiently, we should not only study its propagation and cultivation technology, extraction technology, and application of essential oil but also pay attention to the growth rule of the plant itself. The quantity and quality of plant products depend on whether they are carried out at the optimum period (Dong, 2003). Only by mastering the rule of plant growth and development can we correctly cope with the different requirements of external conditions and management techniques in different periods of plants to achieve greater benefits. This study tracked the transverse and longitudinal diameters, fresh weight of one single fruit and oil yield of *Litsea cubeba* fruit. The purpose is to explore the annual change of fruit traits of *Litsea cubeba* and determine the optimum harvest time and the period with high essential oil content. It is also necessary to clarify the content of essential oils and the change in essential oil components. In-depth research on the annual change of *Litsea cubeba* fruit can provide a theoretical basis for the optimization of its production practice, quality and yield (Forlani et al., 2021).

2. Methods And Materials

2.1 Study area and materials

The experiment was carried out in 2020 in the Key Laboratory of Economic Forest Cultivation and Protection, Ministry of Education, Central South University of Forestry and Technology, Hunan Province. *Litsea cubeba* was planted in 2010 with a height of 2–4 m and a planting density of 4m × 3m. 280 g compound fertilizer (urea 46.4%, superphosphate 18%, potassium carbonate 100%, ratio 3:2:2) was applied to each plant yearly, and weeding was done twice a year. The area is 113° 05 'east longitude and 27° 43' North latitude. Flat hills at an altitude of 90m. The mean annual temperature is about 23°C, the mean annual rainfall is about 1500 mm, and it has a subtropical monsoon climate. The soil is acidic red soil, and the pH is about 5.

2.2 Study instruments and equipment

The main instruments and equipment used in this study are as follows: 1000mL DZTW temperature control heating set (Beijing Yongguangming Medical Instrument Co., LTD.), Clarus 600 gas chromatography-mass spectrometry (Perkin Elmer Instruments Co., LTD.), microwave oven (P70D20N1P-G5 (W0), Galensys, China, 20L), vernier calipers (1/1000cm), electronic analytical balance (1/10000g), thermometer, condensing tube, sorting funnel, conical flask, beaker, round-bottom flask (1L).

2.3 Data collection

2.3.1 Plant sample collection

Fruits in the middle part of the fruiting branch were randomly collected once or twice a month as the fruit samples from the 10th day after flower anthesis (April 3) and collected once a week from the maturity stage (July 5). April 3 is marked as day 0 of fruit development. 200g of fruit was collected from each of the five *Litsea cubeba* plants, and 1000 g of fruit was collected. Stop collecting until the fruit is fully ripe. The collected fruit materials were brought back to the laboratory with crisper boxes for direct experiments or stored at 4 degrees Celsius until use.

2.3.2 Methods for measuring fruit morphological changes

After each sampling, 20 fruit samples with normal development and no pests or diseases were selected by quartering and repeated eight times. The transverse and longitudinal diameters were measured with vernier calipers (precision 1/1000 cm), and the fresh weight was measured with an electronic analytical balance (1/10000 g).

2.3.3 Essential oil extraction method

The essential oil of *Litsea cubeba* was extracted by hydrodistillation. The distillation process was heated with a temperature-controlled heating jacket. Microwave pretreatment places the fruit in a 1000 mL round-bottom flask equipped with a Clevenger device, maintains a liquid-to-material ratio of 10:1, and distillates for three hours.

Essential oil yield calculation formula (%):

$$\text{Yield (\%)} = \frac{\text{Essential oil weight}}{\text{Fruit fresh weight}} \times 100\%$$

2.3.4 Method for determination of essential oil component

0.05mL of fruit essential oil was mixed with anhydrous ethanol in a 10 mL volumetric flask and passed through a 0.22 µm filter membrane, repeated three times, and then detected by GC-MS.

Gas chromatography: Column HP-5MS Quartz capillary column (30 m × 0.25 mm × 0.25 µm). Programmed heating: the initial temperature of the column was 50°C. After 2 min of insulation, the temperature was raised to 125°C at a rate of 3°C/min and maintained for 1min, and then the temperature was raised to 250°C at a rate of 15°C/min for 5min. Carrier gas: high-purity helium (99.999%). The precolumn pressure, flow rate, injection volume, scanning time, injection port temperature and split ratio were 50 kPa, 1 mL/min, 1 µL, 4 min, 220°C and 1:40, respectively.

Mass spectrometry: the ion source is an EI-source, the ionization voltage is 70 eV, the quality scan range is 30–550 amu, the quadrupole temperature is 150°C, the ion source temperature is 230°C, and the interface temperature is 260°C.

2.4 Data analysis

IBM SPSS Statistic 23 was used for data analysis. One-way analysis of variance (ANOVA) was used to analyze the data obtained from the three one-way experiments statistically, and the least significant difference (LSD) was used to determine the significant difference between the means ($P < 0.05$). Pearson correlation analysis was used to test the correlation between variables. Draw charts with Origin 2021 64bit software.

3. Results

3.1 Annual change of fruit morphology of *Litsea cubeba*

3.1.1 Changes of transverse diameter and longitudinal diameter in *Litsea cubeba* fruit.

The growth curve (Fig. 1, a) of the mean transverse and longitudinal diameter of *Litsea cubeba* fruit showed a tendency of rapid growth in the early stage, slow growth in the middle stage, rapid growth to the highest in the mid to late stage, and rapid decline in the late stage. The fruit diameter growth trend of *Litsea cubeba* can be divided into four stages: the first stage (from day 0 to day 54, lasting 54 days) was the rapid growth stage, and the fruit diameter increased at the fastest rate in this stage. The growth amount reached 0.34 cm, accounting for 66.57% of the total growth amount, and the growth rate reached 0.0063 cm/d. The second stage (from day 54 to day 103, lasting 49 days) was the slow growth stage, the growth of fruit diameter at this stage was the gentlest in the whole fruit period. The fruit diameter increased by 0.061 cm, accounting for only 12.16% of the total growth. The third stage (from day 103 to day 132, lasting 29 days) was the

second rapid growth stage, and the *Litsea cubeba* fruit began to enter the mature period. The fruit diameter increased from 0.54 cm to the maximum value of 0.65 cm, an increase of 0.11 cm, accounting for 21.26% of the total growth. The fourth stage (from day 132 to day 154, lasting 22 days) was the rapid reduction stage. Since the fruit began to lose water and shrivel, the diameter decreased rapidly, reducing the total growth by 6.60%, and the decreasing rate was 0.0015 cm/d.

It can be seen from Fig. 1(b) that the mean transverse and longitudinal diameter of *Litsea cubeba* fruit still increased slowly after entering the maturity period. From day 115 to day 146 (from July 27 to August 27), the fruit diameter reached the maximum value of 5.97mm, and its growth changed significantly. Then the fruit began to shrivel, and the fruit diameter began to decrease. The fruit diameter decreased by 0.09 mm from day 146 to day 154 (from August 27 to September 4).

The fitting curves in Fig. 1 can generally simulate the dynamic change of the mean transverse and longitudinal diameter of *Litsea cubeba* fruit at whole fruiting stage(a) and at fruit maturity period(b), and the fitting equations are as follow:

(a)

$$Y = 1.4711 \times 10^{-1} \pm 1.06 \times 10^{-2} + 3.48847 \times 10^{-4} \pm 1.61 \times 10^{-3} x + 4.33974 \times 10^{-4} \pm 7.15258 \times 10^{-5} x^2 \\ + -9.20981 \times 10^{-6} \pm 1.25393 \times 10^{-6} x^3 + 7.10432 \times 10^{-8} \pm 9.30665 \times 10^{-9} x^4 \\ + -1.86924 \times 10^{-10} \pm 2.44709 \times 10^{-11} x^5$$

(b)

$$Y = 5.10451 \pm 2.42844 + 1.0585 \times 10^{-1} \pm 5.462 \times 10^{-2} x + 8.16259 \times 10^{-4} \pm 4.07541 \times 10^{-4} x^2 \\ + -2.07335 \times 10^{-6} \pm 1.00866 \times 10^{-6} x^3$$

3.1.2 Changes of the fresh weight in one single *Litsea cubeba* fruit

As can be seen from Fig. 2(a), the growth of fruit fresh weight of *Litsea cubeba* showed a trend of rapid growth at first, slow growth in the middle, rapid growth to the highest in the mid to late, and finally rapid decline. According to the trend of fruit weight, *Litsea cubeba* could be divided into four stages. The first stage (from day 0 to day 54, lasting 54 days) was the rapid growth stage, and the fruit weight increased from 0.0019 g on April 3 to 0.065 g on May 27, which increased 34 times. The second stage (from day 54 to day 103, lasting 49 days) was a slow growth stage, and the growth rate of fruit weight in this stage was only 0.001 g/d. The third stage (from day 103 to day 132, lasting 29 days) was the second rapid growth stage, at which *Litsea cubeba* fruit ripened and fruit diameter increased rapidly again. Fruit weight increased to the maximum value and the growth rate reached 0.002 g/d, which was twice that of the slow growth stage. The fourth stage (from day 132 to day 154, lasting 22 days) was the rapidly decreasing stage, and the *Litsea cubeba* fruit began to lose water and shrivel. Fruit weight decreased by 15.62% of the total growth.

As shown in Fig. 2(b), the fresh weight of *Litsea cubeba* fruit increased slowly after entering the maturity stage. From day 115 to day 146 (from July 27 to August 27), the average fresh weight was 0.1321g, which was 14.4% higher than that of the day 115 (July 27). Then the fruit entered the shriveling stage, and the fruit weight gradually decreased. The fruit weight decreased by 0.0025g from day 146 to day 154 (from August 27 to September 4), with no significant difference.

The fitting curves in Fig. 2 can generally simulate the dynamic change of fruit weight of *Litsea cubeba* fruit at whole fruiting stage(a) and at fruit maturity period(b), and the fitting equations are as follow:

(a)

$$Y = 2.53 \times 10^{-3} \pm 3.88 \times 10^{-3} + -1.46 \times 10^{-3} \pm 5.8823 \times 10^{-4} x + 1.47723 \times 10^{-4} \pm 2.61873 \times 10^{-5} x^2 \\ + -2.83472 \times 10^{-6} \pm 4.59091 \times 10^{-7} x^3 + 2.23777 \times 10^{-8} \pm 3.40738 \times 10^{-9} x^4 \\ + -6.19257 \times 10^{-11} \pm 8.95936 \times 10^{-12} x^5$$

(b)

$$Y = 1.29302 \pm 1.96756 + -2.841 \times 10^{-2} \pm 0.04439 x + 2.23978 \times 10^{-4} \pm 3.32166 \times 10^{-4} x^2 \\ + -5.74928 \times 10^{-7} \pm 8.24374 \times 10^{-7} x^3$$

3.2 Annual change of the essential oil content in *Litsea cubeba* fruit

3.2.1 Changes of essential oil content in *Litsea cubeba* fruit

As can be seen in Fig. 3(a), the annual cycle trend of essential oil content in *Litsea cubeba* fruits was first slow growth, then rapid growth, and rapid decline. The developmental process can be divided into three stages: in the first stage (from day 0 to day 62), the essential oil accumulation was slow, and the average daily increase was less than 0.01%. The essential oil content of *Litsea cubeba* fruit was almost 0 in the early stage of development, but after 54 days of slow accumulation, it increased to 0.46%. The growth in the first stage accounted for 17.70% of the total growth. In the second stage (from day 62 to day 103), the essential oil content of *Litsea cubeba* fruits increased rapidly, and the essential oil content increased from 0.46–2.60%, accounting for 82.30% of the total increase. The essential oil content decreased rapidly in the third stage (from day 103 to day 154). The essential oil content of *Litsea cubeba* fruit decreased from 2.60–1.68%, with a decrease of 0.92%, accounting for 35.51% of the highest amount.

It can be seen from Fig. 3(b) that after entering the maturity period, the essential oil content of *Litsea cubeba* fruits showed four obvious stages: rapid increase to the highest in the early stage, rapid decrease in the middle stage, the decreasing trend slows down in the mid to late stage, and rapid decrease secondly in the later stage. In the rapid growth stage (from day 93 to day 121), the essential oil content of fruits increased rapidly, and the growth changes were significantly different. It reached the highest value on August 2, with

the essential oil content up to 3.88%. In the first rapid decreasing stage (from day 121 to day 129), the essential oil content of fruits decreased rapidly, and the difference was significant. The essential oil content of fruits decreased to 2.71%, which was only 69.85% of the original content. In the slow decline stage (from day 129 to day 146), the essential oil content decreased by 0.34%, and there was no significant difference. In the second rapid decreasing stage (from day 146 to day 154), the essential oil content of *Litsea cubeba* fruit decreased rapidly again, from 2.37–1.23%, only 51.90% of the original, and the difference was significant.

The fitting curve in Fig. 3 can generally simulate the dynamic change of the essential oil content of *Litsea cubeba* fruit at whole fruiting stage(a) and at fruit maturity period(b), and the fitting equations are as follow:

(a)

$$Y = \frac{3.006 \times 10^{-2} \pm 6.42 \times 10^{-2} + (2.66 \times 10^{-3} \pm 9.77815 \times 10^{-4})x}{(1 + -1.64 \times 10^{-2} \pm 3.5642 \times 10^{-4} x + 7.66683 \times 10^{-5} \pm 3.65229 \times 10^{-6} x^2)}$$

(b)

$$Y = -26.01764 \pm 5.72571 + -4.7641 \times 10^{-1} \pm 8.835 \times 10^{-2} x + -1.94 \times 10^{-3} \pm 3.35522 \times 10^{-4} x^2$$

3.3 Changes of essential oil components in *Litsea cubeba* fruit

3.3.1 Changes of essential oil components type in *Litsea cubeba* fruit

Through GC-MS analysis of the essential oil of *Litsea cubeba* fruit at different stages, it can be seen that there were significant differences in the essential oil components at different development times. The difference is mainly reflected in the main components and the total number of components. As Table 1, day 115 (July 27) has the maximum number of components, with 29 species. Furthermore, day 129 (August 10) has the minimum number of components, with only 18 species. The main components in each period contained α -citral, β -citral, and D-limonene. Palmitic acid was the main component only on day 93 (July 5) and day 121 (August 2), with a relative content of more than 2%. Eudesmol was the main component only on day 109 (July 21), day 115 (July 27) and day 154 (September 4), with a relative content of more than 2%. Citronellal became a main component after day 139 (August 20). In addition, the relative content of pentadecanoic acid was higher than 2% only on day 93 (July 5).

Table 1
Change of essential oil components of *L. cubeba* fruit with fruit growth

Fruit develop- ment time	Number of compon-ents	Main components	Proportion of total peak area /%
93	28	α -citral β -citral Stearic acid Palmitic acid Pentadecanoic acid D-limonene	89.25
109	23	α -citral β -citral D-limonene Eudesmol	89.51
115	29	α -citral β -citral D-limonene Eudesmol	84.21
121	22	α -citral β -citral D-limonene Palmitic acid	87.71
129	18	α -citral β -citral D-limonene	90.25
133	23	α -citral β -citral D-limonene	89.63
139	24	α -citral β -citral D-limonene Citronellal	90.93
146	21	α -citral β -citral D-limonene Citronellal	89.18
154	26	α -citral β -citral D-limonene Eudesmol Citronellal	91.15
Note: The names of main components are arranged from left to right, and the relative content is from high to low. The main components are those with relative content above 2%.			

3.3.2 Changes of relative contents of essential oil components in *Litsea cubeba* fruit

The relative contents of the main components of essential oil of *Litsea cubeba* fruit changed with fruit development time, as shown in Table 2. Citral has the highest relative content during fruit growth. The lowest citral content reached 58.36% on day 93 (July 5), and the highest citral content reached 85.14% on day 139 (August 20). On the 93rd day of fruit development, the relative contents of stearic acid, palmitic acid and pentadecanoic acid were 15.62%, 14.46% and 6.21%, respectively. The relative contents of stearic acid, palmitic acid and pentadecanoic acid decreased rapidly with the increase in growth time. The relative contents of stearic acid and pentadecanoic acid were below 2%, and palmitic acid was above 2% only on day 121 (August 2). In addition, citral and D-limonene were the main components during the whole period. Citronellal began to increase to more than 2% on day 139.

Table 2

Changes of relative contents of main components of *L. cubeba* fruit essential oil with fruit growth

Fruit develop-ment time	Main components					
	Relative content(%)					
93	α -citral	β -citral	Stearic acid	Palmitic acid	Pentadecanoic acid	D-limonene
	29.18	21.29	15.62	14.46	6.21	2.49
109	α -citral	β -citral	D-limonene	Eudesmol		
	47.85	35.05	3.91	2.7		
115	α -citral	β -citral	D-limonene	Eudesmol		
	43.03	32.88	5.52	2.78		
121	α -citral	β -citral	D-limonene	Palmitic acid		
	45.43	33.6	4.7	3.98		
129	α -citral	β -citral	D-limonene			
	48.27	36.27	5.71			
133	α -citral	β -citral	D-limonene			
	48.29	35.63	5.71			
139	α -citral	β -citral	D-limonene	Citronellal		
	49.04	36.1	3.6	2.19		
146	α -citral	β -citral	D-limonene	Citronellal		
	47.15	35.1	4.73	2.2		
154	α -citral	β -citral	D-limonene	Eudesmol	Citronellal	
	46.99	34.7	4.48	2.51	2.47	
Note: The components are arranged from left to right and the relative content is from high to low, the components that more than 2% relative content are considered as main components.						

3.4 Correlation between fruit traits and developmental time of *Litsea cubeba*

The Bivariate Pearson test was used to explore the correlation between the duration of fruit development and fruit morphology of *Litsea cubeba*. As shown in Table 3: the duration of fruit development was strongly positively correlated with the fruit diameter, single fruit fresh weight and oil content. The correlation coefficient of experimental samples could represent the overall correlation degree ($P < 0.01$). The correlation coefficient with single fruit fresh weight was the highest, which was 0.973, the fruit diameter was 0.907, and the smallest was oil content, which was 0.851.

Table 3
Correlation between transverse diameter, longitudinal diameter, fresh weight of single fruit and the oil content and fruit growth time

Duration of fruit development		Correlation coefficient
Factors	Sig.	
Fruit diameter	0.000	0.907**
Single fruit fresh weight	0.000	0.973**
Oil content	0.000	0.851**
Note: "*" means $P < 0.05$, "***" means $P < 0.01$.		

3.5 Correlation between morphological changes and essential oil accumulation in *Litsea cubeba* fruit

As Table 4, the Bivariate Pearson test was used to explore the correlation between fruit morphology and essential oil accumulation in *Litsea cubeba*. There was a significant negative correlation between fruit morphology and essential oil accumulation. The correlation coefficient of experimental samples could represent the overall correlation degree ($P < 0.05$). The correlation coefficient was -0.693 .

Table 4
Correlation between fruit morphological changes and essential oil accumulation

	Sig.	Correlation coefficient
Essential oil content growth rate	0.038	-0.693*
Fruit diameter growth rate		
Note: "*" means $P < 0.05$, "***" means $P < 0.01$.		

4. Discussion

The Bivariate Pearson test (Table 4) indicates a significant negative correlation between morphological changes and essential oil accumulation of *Litsea cubeba* fruit. Informed research has shown that the essence of *Litsea cubeba* fruit growth can be summarized as the division and expansion of fruit cells, accumulation and transformation of substances (Wang et al., 2007). The analysis was carried out by

combining the curve of annual changes in fruit diameter and weight of *Litsea cubeba* (Figs. 1 and 2, a) and the curve of annual changes in fruit essential oil accumulation of *Litsea cubeba* (Fig. 3, a). It can be found that the fruit cell division is rapid during the early stage, resulting in the rapid growth of fruit diameter and weight. In contrast, essential oil accumulation is relatively slow in the early development stage. This phenomenon is caused by the fact that most of the nutrients supplied to the fruit are used for the division of the fruit cells. In the middle stage, the speed of fruit cell division slowed down, which slowed the growth rate of fruit diameter and weight but increased the accumulation rate of essential oil. With the stop of cell division, *Litsea cubeba* fruit entered the later stage of development, and the expansion of fruit cells began. As fruit cell division stops, the essential oil gradually stops accumulating and the content decreases. With the expansion of cells, fruit diameter and weight increased rapidly until the maximum value of the whole fruit period. It is obvious that plant nutrients are mainly used for fruit expansion in the later stage of fruit development. *Litsea cubeba* fruit reaches the end stage of development after full maturity. The fruits began to shrivel, decreasing the fruit diameter and weight rapidly in a short time, and the essential oil content continued to decrease. In conclude, plant nutrient allocation may determine the relationship between fruit morphology and essential oil accumulation in *Litsea cubeba*.

By analyzing the annual change curve of *Litsea cubeba* fruit, we can find that essential oil accumulation follows a single "S" curve with slow growth at first, then rapid growth, and rapid decrease (Fig. 3, a). The morphological development of *Litsea cubeba* fruit (fruit mean transverse and longitudinal diameter and fruit weight) showed an obvious tendency of a double "S" curve with rapid growth at first and followed by slow growth, and finally rapid growth again (Fig. 1, a). The morphological development characteristics of plants such as Longan (Peng et al., 2021), Betula (Liu, 2013), and Gloriosa (Chen, 2008) are similar to those of *Litsea cubeba*, and they all conform to the double "S" curve. However, the development of *Litsea cubeba* at each period needs to be combined with the growth curve of fruit maturity. The growth and development process of *Litsea cubeba* fruit can be divided into four periods according to the annual cycle growth curve and maturity stage growth curve of fruit diameters and fruit weight (Figs. 1 and 2, a): the first rapid growth period (day 0 to day 54, April 3 to May 27), the slow growth period (day 54 to day 121, May 27 to August 2), the second rapid growth period (day 121 to day 146, August 2 to August 27), and the shriveling period (day 146 to day 154, August 27 to September 4). The fruit diameter and fruit weight reached the maximum value on August 27. By combining the annual cycle growth curve (Fig. 3, a) and the maturity stage growth curve (Fig. 3, b) of the fruit essential oil accumulation, the growth and development process of the fruit can be divided into three periods: the slow accumulation period (day 0 to day 54, April 3 to May 27), the rapid accumulation period (day 54 to day 121, May 27 to August 2), and the decreasing period (day 121 to day 154, August 2 to September 4). Among them, the decreasing period can be divided into three phases: the first rapid decreasing phase (day 121 to day 129, August 2 to August 10), the slow decreasing phase (day 129 to day 146, August 10 to August 27), and the second rapid decreasing phase (day 146 to day 154, August 27 to September 4). The essential oil content reached the highest on day 121 (August 2). Combining the periods of fruit morphology and essential oil accumulation, the growth and development of *Litsea cubeba* fruit can be divided into four general periods: the first rapid growth period (day 0 to day 54: rapid growth in fruit diameter and fruit weight with slow accumulation of essential oil), the essential oil accumulation period (day 54 to day 121: rapid accumulation of essential oil with slow growth in fruit

diameter and fruit weight), the second rapid growth period (day 121 to day 146: fruit diameter and fruit weight increased rapidly for the second time while the essential oil content started to decrease), and the shriveling period (day 146 to day 154: fruit diameter and weight and essential oil content all decreased rapidly). These four periods summarize the fruit annual change rule of *Litsea cubeba*, which is directly helpful to production practice.

The preliminary test results showed that the maturity stage was the main stage of essential oil production, so the essential oil components in this stage were detected. Tables 1 and 2 show the results of GC-MS analysis of essential oils of *Litsea cubeba* fruits at the maturity stage (day 93 to day 154). According to Table 2, the component with the highest content of essential oil in maturity stage was citral, which is consistent with most previous studies (Zhou et al., 2020; Dalimunthe et al., 2021; Nguyen et al., 2018). The citral content was low on day 93 (July 5), then increased rapidly and stabilized. Therefore, we can conclude that the citral content was low to high from day 0 to day 93, and the essential oil of *Litsea cubeba* fruit has the same trend at this stage. From day 129 to day 139 (August 10 to August 20), the average relative content of citral was about 84.5% and the highest was 85.14% (August 20, day 139). Therefore, when citral extraction is needed, fruits can be harvested from day 129 to day 139 of *Litsea cubeba* fruit development. In this research, the relative content of *D-Limonene* in the essential oil of *Litsea cubeba* fruit was kept at a low level of 2.49–5.71%. Different to this study, in previous studies (Lan et al., 2020), *D-Limonene* in the essential oil of *Litsea cubeba* fruits has a high relative content, which is even higher than the content of citral at the early stage, and its content gradually decreases with fruit development. In addition, at the beginning of the maturity period (day 93, July 5), there are lots of acids detected in the fruit essential oil, such as stearic acid (15.62%), palmitic acid (14.46%) and hexadecanoic acid (6.21%). However, the acid content decreased rapidly with fruit development, and in the middle stage of fruit development (day 109, July 21) the content was below 2%. These indicated that acids were consumed with the growing development of *Litsea cubeba* fruit. Liu (2013) studied the development of sandalwood fruit and its essential oil accumulation rule. He found that acids such as palmitic acid in sandalwood fruit oil were significantly negatively correlated with the morphological indexes such as fruit dry and fresh weight, transverse diameter and longitudinal diameter, and stearic acid was significantly negatively correlated with fruit dry weight, which was consistently with the results of this study.

Fruit essential oil has been extensively studied because of its high application and economic value. Based on this study on the accumulation rule of essential oil of *Litsea cubeba*, the factors promoting the accumulation of essential oil of *Litsea cubeba* can be explored in the future. The dynamic formation and accumulation of fruit essential oils are affected by physiological and geographical changes, genetics and environmental factors (Zhang et al., 2020; Huang et al., 2021). The correlation analysis by Yuan et al. (2021) showed that the essential oil content of *Litsea cubeba* fruit was significantly negatively correlated with the longitude of origin, and mean temperature in July, negatively correlated with annual rainfall and positively correlated with altitude. Huang et al. (2020) proved through the experiment that the microbial agents could effectively promote the growth of *Cinnamomum camphora* and the accumulation of essential oil. In addition, previous studies have shown that environmental factors such as soil moisture (Souza et al., 2021), fertilizer (Sabzi-Mehrabad et al., 2018), volatile bacterial elicitors (Banchio et al., 2009), and volume of the fruit cavity (Liu et al., 2019) can affect the accumulation of essential oil. The research on the factors

affecting essential oil accumulation of *Litsea cubeba* needs to be more comprehensive, which is worth further exploration from the above aspects.

5. Conclusion

In this study, the annual changes in fruit morphology and essential oil of *Litsea cubeba* fruit were investigated by covering sampling at the whole fruit period. The essential oil was extracted by hydrodistillation, and its chemical components were analyzed and identified by GC-MS. The relationship between the growth of fruit morphology and essential oil accumulation was discussed and analyzed, and the optimal harvest time was also determined. The annual change of fruit morphology of *Litsea cubeba* was consistent with the double "S" curve, which shows a general trend of rapid growth first, then slow growth, and finally rapid growth again. While the annual change of the essential oil of *Litsea cubeba* fruits was consistent with a single "S" curve, which shows a general trend of slow growth first, then rapid growth, and finally decreasing rapidly. Correlation analysis showed a significant negative correlation between morphological changes and essential oil accumulation in *Litsea cubeba* fruit. And this may be caused by the plant nutrient allocation. Combining the growth curves of fruit morphology and fruit essential oil, the growing development of *Litsea cubeba* fruit can be divided into four general periods: the first rapid growth period, the essential oil accumulation period, the second rapid growth period, and the shriveling period. The diameter and weight of *Litsea cubeba* fruit reached the maximum on day 146 (August 27), and the essential oil content of *Litsea cubeba* reached the maximum on day 121 (August 2). It was also confirmed that citral was the highest content component in the essential oil of *Litsea cubeba*. The maximum content reached 85.14% (day 139, August 20). This result will provide theoretical support for the efficient production and application of *Litsea cubeba* fruit. Furthermore, this study found that the acid components in the essential oil of *Litsea cubeba* were gradually consumed. In contrast, the aldehydes components increased with the development of *Litsea cubeba* fruit, and the changing mechanism needs further study.

Declarations

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Data availability

Datasets generated during and/or analyzed in the current study are available from the corresponding author on request.

Conflict of interest

Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All authors have read the MS and approved.

Ethics approval and consent to participate

The plant material is derived from the Central South University of Forestry and Technology woody spice plant base. From picking to extraction, all methods are carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable

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Figures

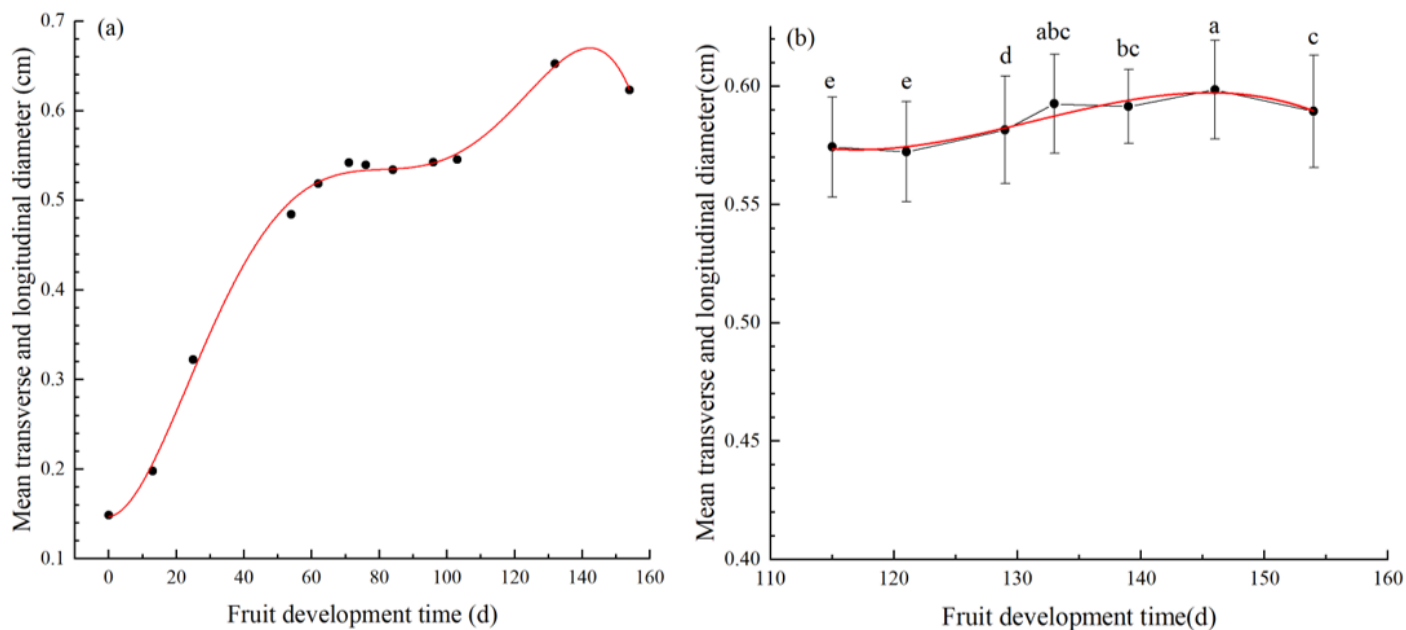


Figure 1

(a) Dynamic change of the mean value of transverse and longitudinal diameter of *L. cubeba* at whole fruiting stage and (b) at fruit maturity period.

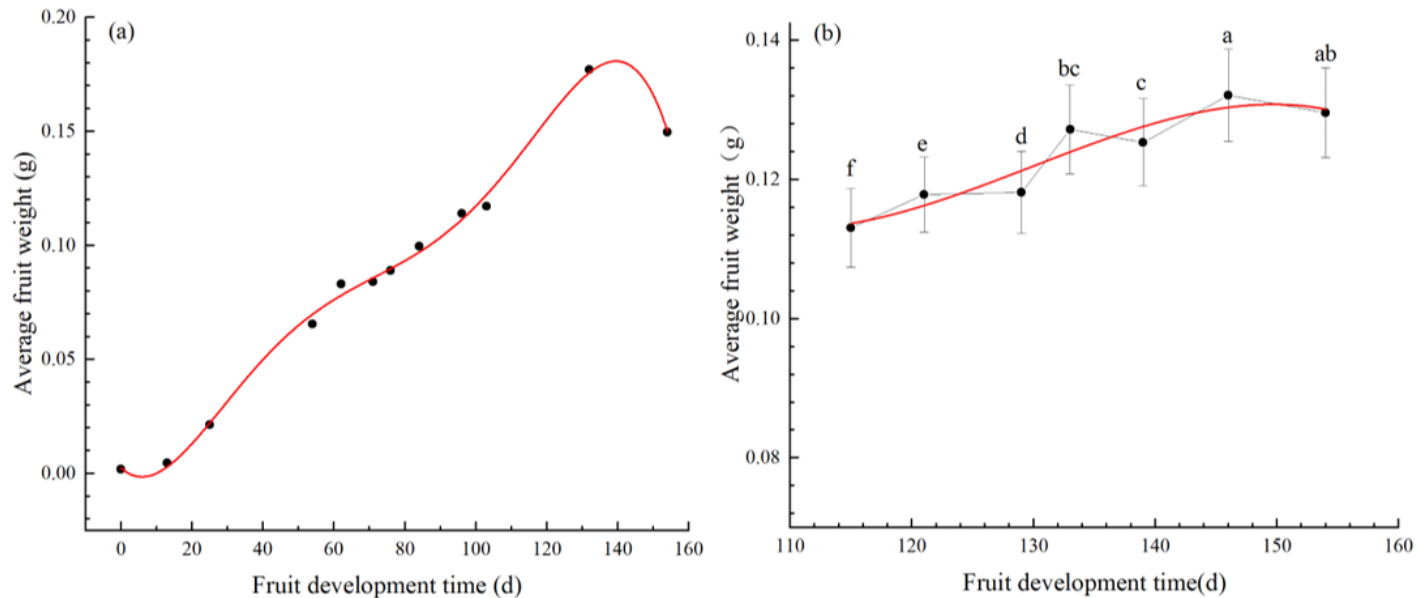


Figure 2

(a) Dynamic change of the fresh weight of one single *L. cubeba* fruit at whole fruiting stage and (b) at fruit maturity period.

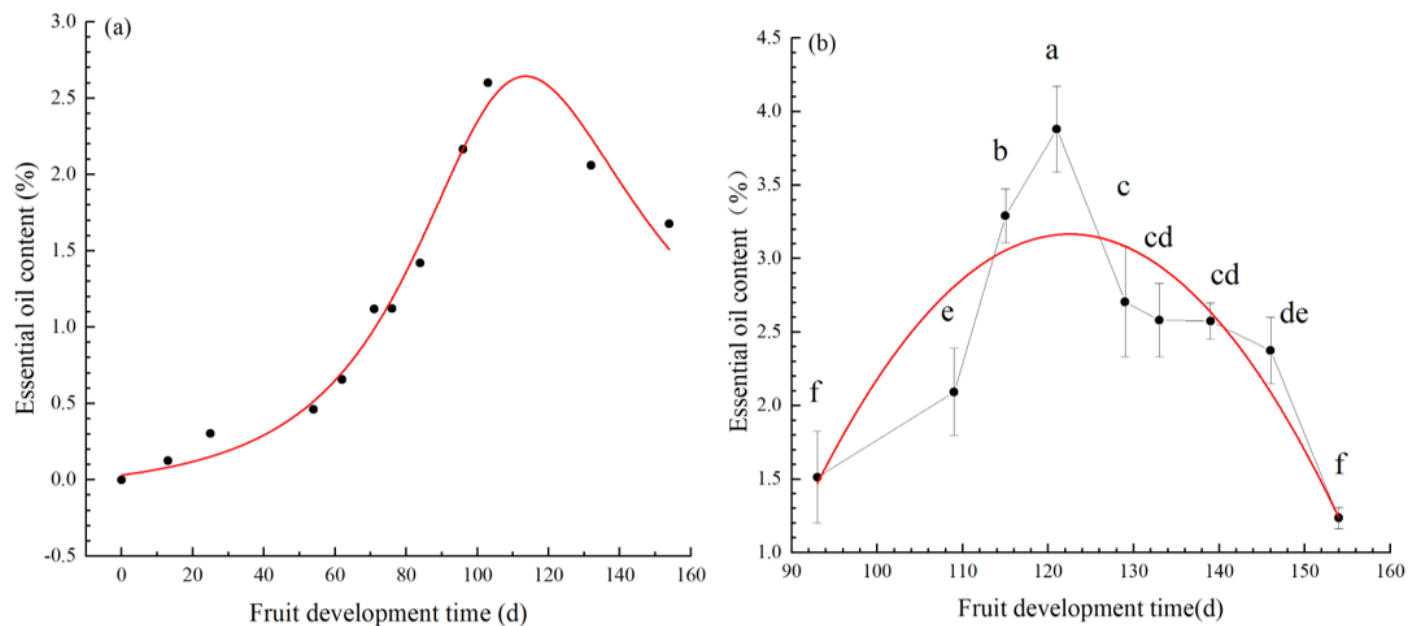


Figure 3

(a) Dynamic change of the essential oil content of *L. cubeba* fruit at whole fruiting stage and (b) at fruit maturity period.