Optimization of Process Conditions for Extracting *Litsea cubeba* Kernel Oil by Microwave-Assisted Water Method

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Abstract. *Litsea cubeba* is a rich woody oil plant resource in China. The economic value of *Litsea cubeba* oil is high. In order to improve the resource utilization rate of *Litsea cubeba*, this study used microwave-assisted water method to extract *Litsea cubeba* kernel oil from the seeds of *Litsea cubeba*. The optimum process conditions were as follows: microwave time 60 min, extraction temperature 80.18 °C, liquid-to-liquid ratio 11.46:1, microwave power 587.44 W. Under this optimized condition, the extraction rate of *Litsea cubeba* kernel oil was 37.54%. It can be seen that the microwave assisted water generation method for extracting *Litsea cubeba* nucleolus oil is reliable and has certain utilization value.

1. Introduction
*Litsea cubeba* (Lour.) Pers. is a kind of deciduous shrub and is native to China, Indonesia and other parts of southeast Asia[1]. The fruit is known in the traditional Chinese medicine as an antibacterial, antifungal and anti-inflammatory agent which contains about 4% to 8% essential oil and 20% to 50% kernel oil[2-4]. A large number of kernel residues will be simultaneously produced during the extraction of LC essential oils. Although some companies have tried to use by-products or so-called waste to make medical pillows or to extrude oil for added value, due to lack of knowledge, unclear technology and unprofitable output, these by-products or so-called wastes are not fully utilized[5]. The large amount of kernel discarded not only causes the low utilization rate of the raw material of *Litsea cubeba*, but also causes serious pollution to the local ecological environment.

Water method is a traditional method in China. Water extraction technology requires less equipment, low investment efficiency, and does not need to consider the problem of organic solvent residue. It is a more efficient and environmentally friendly extraction method[6,7]. In order to make full use of the resources of *Litsea cubeba*, this study used microwave-assisted water method to extract *Litsea cubeba* oil, and designed response surface analysis to optimize its extraction process.

2. Materials and methods

2.1 Experimental materials and reagents
The seed of *Litsea Cubeba* (Lour.) Pers. were supplied by Yongzhou Samshiang Spice Co., Ltd. and were ground through a set of standard size sifter to obtain the powder samples and sifted successively; Phosphoric acid (AR, Sinopharm Chemical Reagent Co., Ltd.); Acetic acid (AR, Sinopharm Chemical Reagent Co., Ltd.).
2.2 Experimental Methods
The raw material of Litsea cubeba nucleolus is screened, pulverized and ground, accurately weighed, placed in the reaction kettle, and added with a certain amount of pure water for homogenization, adjusted to a suitable pH and heated and stirred, and then placed in a microwave digestion apparatus. In the microwave extraction, the extract is subjected to high-speed centrifugation, and the supernatant is taken for rotary evaporation to remove water, and the oil is weighed to obtain Litsea cuben kernel oil. The yield of litsea cubeba Kernel oil is calculated as follows:

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\text{Kernel oil yield} = \frac{\text{The quality of kernel oil}}{\text{The quality of Litsea cubeba power}} \times 100\%. \quad (1)
\]

3. Results and discussion

3.1 Single factor test of Microwave-assisted extraction of Lisea Cubeba Kernel oil

3.1.1 The influence of ratio of liquid to solid. Reaction conditions: the raw material mass is 100g, the microwave reaction time is 60 min, the microwave extraction temperature is 85° C, and the microwave power is 400W. Fig. 1 indicated that the Kernel oil yield increased with the ratio, when the ratio of liquid to solid was less than 10: 1. When the ratio of liquid to material increased on the basis of 10: 1, the extraction rate decreased. This is because water can replace the grease in the slurry when the liquid ratio is appropriate. When the amount of water is too small, the viscosity of the slurry will be large, so that the oil is not easily replaced; when there is too much water, the mixed oil and the residue will be easily emulsified.

3.1.2 The influence of Microwave power. Reaction conditions: the raw material mass is 100g, the microwave extraction temperature is 85° C, and the liquid to material ratio is 10: 1. Fig. 2 shows the effect of Microwave power on extraction of Litsea cubeba Kernel oil. Within 600W, the extraction rate of Litsea cubeba kernel oil increased with the increase of microwave power, but after more than 600W, the extraction rate decreased with the increase of power. This is mainly because the proper increase of microwave power can increase the damage to cells and promote the release of oil. However, when the microwave power exceeds the critical value, it will not only affect the destruction of oil molecules, but also cause the emulsification of oil and cell components, thereby reducing the extraction rate of oil.

3.1.3 Effect of Microwave time. Reaction conditions: the raw material mass is 100g, the microwave extraction temperature is 85° C, the microwave power is 600 W, and the liquid to material ratio is 10: 1. As shown in Fig. 3, when the extraction duration was less than 65min, the oil yield increased with the rising of time. On the contrary, the yield reduced a little after 65min. This may be due to the decomposition of the oil caused by overlong extraction time.

3.1.4 Effects of temperature on the products yield. Reaction conditions: the raw material mass is 100g, the microwave power is 600 W, the liquid to material ratio is 10:1 and the microwave reaction time is 65 min. As inferred from Fig. 4, when the microwave extraction temperature is within 70-80 °C, the extraction rate of Litsea cubeba oil is obviously increased with the increase of extraction temperature; when the extraction temperature is higher than 80 °C, the extraction rate decreases with the increase of extraction temperature. This is because at low temperatures, the viscosity of the grease is large and the water and oil are difficult to delaminate. With the increasing of the temperature , it could enhance the molecular motion and is beneficial to cavitation, that can cause a result of an increased diffusivity of the solvent into cells and an enhanced desorption of the oil from cells[8, 9].
3.2 Response surface optimization experiment

On the basis of the results of single factor experiments, three factors with large influences of microwave power, microwave temperature and liquid-to-material ratio were selected as the investigation factors. The response surface design was carried out by Box-Behnken Design[10,11]. The horizontal coding of test factors is shown in Table 1. The fitting analysis was performed based on the experimental data of Table 1, and the results are shown in Table 2.

| Independent variables | Microwave power (A)/W | Temperature (B)/℃ | Liquid-to-solids ratio (C) |
|-----------------------|-----------------------|-------------------|---------------------------|
| -1                    | 500                   | 75                | 5.00                      |
| 0                     | 600                   | 80                | 10.00                     |
| 1                     | 700                   | 85                | 15.00                     |

Table 2. Experimental design for three variables with CCD method

| Std. order | A  | B  | C  | Extraction rate/(%) |
|------------|----|----|----|----------------------|
| 1          | -1 | 1  | 0  | 32.1                 |
| 2          | 1  | 1  | 0  | 31.6                 |
| 3          | 0  | 0  | 0  | 30.1                 |
| 4          | 0  | 0  | 0  | 30.1                 |
| 5          | 0  | -1 | 1  | 16.9                 |
| 6          | 0  | 1  | 1  | 36.4                 |
| 7          | 0  | -1 | -1| 37.1                 |
Using the Design-expert8.0.6 software to fit the test results, we can obtain the quadratic multiple regression model for the extraction rate of Litsea cuben kernel oil to A, B and C:

$$Y = 36.54 - 0.82A + 0.16B + 6.46C - 0.33AB + 0.87AC - 0.09BC - 2.30A^2 - 2.31B^2 - 10.86C^2.$$  (2)

| Source | Squares | df | Square | F   | P-value | Significant |
|--------|---------|----|--------|-----|---------|-------------|
| A      | 10.89   | 1  | 10.89  | 12.78 | 0.0015  | Significant |
| B      | 0.39    | 1  | 0.39   | 0.46 | 0.5048  | Insignificant |
| C      | 666.93  | 1  | 666.93 | 782.70 | <0.0001 | Significant |
| AB     | 0.85    | 1  | 0.85   | 0.99 | 0.3293  | Insignificant |
| AC     | 6.13    | 1  | 6.13   | 7.19 | 0.0131  | Significant |
| BC     | 0.061   | 1  | 0.061  | 0.072 | 0.7909  | Insignificant |
| A2     | 44.60   | 1  | 44.60  | 52.34 | <0.0001 | Significant |
| B2     | 45.08   | 1  | 45.08  | 42.91 | <0.0001 | Significant |
| C2     | 993.86  | 1  | 993.86 | 1166.38 | <0.0001 | Significant |
| Residual | 20.45 | 24 | 0.85   |  |         |             |
It can be seen from Table 3 that the model F value is 238.28 and the model P is smaller than 0.0001, indicating that the regression equation has good fit and the experimental error is small. The above data indicates that the model has high fitness and reliability. This model can be used to analyze and predict the process conditions for extracting the nucleolus oil reaction.

According to the results of regression analysis, the response surface is obtained (Fig. 5-7). It can be seen from Fig. 5 that the extraction rate increases with the increase of liquid-to-solid ratio, but the extraction rate changes less obviously with the increase of microwave power. From the Fig. 7, it can be seen from the density of the contour line that the density of movement along the liquid to material ratio is greater than the microwave temperature, which indicates that the liquid material has a greater influence on the extraction rate. Similarly, the degree of influence of each factor on the extraction rate is consistent with the F value.

The optimal extraction conditions obtained by the response surface test were: liquid-to-solid ratio of 11.46:1, microwave power of 587.41W, and microwave temperature of 80.19 °C. The optimal simulation value under this condition was 37.53%. In order to test the reliability of the optimization results, three repeated experiments were carried out, and the average extraction rate was 36.47%, which was close to the simulated value. It indicated that the process condition of optimizing the
micro-assisted water generation method to extract *Litsea cubeba* oil by response surface method is reliable.

### 3.3 Determination of fatty acid composition

Under the microwave-assisted water method for extracting the nucleolus oil of *Litsea cubeba*, the fatty acid composition of the oil was determined according to the national standard (GB/T 17376-2008) using boron trifluoride methyl ester method. The results are shown in Fig. 6.

![Fig. 8 Fatty acid composition of *Litsea cubeba* kernel oil](image)

It can be seen from Fig. 8 that the five kinds of saturated fatty acids and five kinds of unsaturated fatty acids are identified in the *Litsea cubeba* kernel oil extracted by the microwave-assisted water method. The content of saturated fatty acid C12: 0 (lauric acid) is more than 50%, the content of C10: 0 (capric acid) is 8.512%, and the content of unsaturated fatty acid C18: 1 (oleic acid) is 10.603%, which is consistent with the fatty acid composition of *Litsea cubeba* kernel oil.

### 4. Conclusions

The single-factor test and Box-behnken experimental design were used to optimize the extraction process of *Litsea cubeba* oil. The optimal process conditions were microwave power 545W and microwave temperature 80.19°C, when the ratio of material to liquid is 11.46: 1, the extraction rate of *Litsea cubeba* oil is 37.54%. Through the analysis of fat components in oils, the microwave-assisted water-based extraction of *Litsea cubeba* kernel oil is in line with the components of *Litsea cubeba* seeds. It indicated that the process condition of optimizing the micro-assisted water method to extract *Litsea cubeba* oil by response surface method is reliable.

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