Optimization of aerobic composting process of Camallia oleifera Seed Cake and its fertilizer efficiency

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Abstract. [Objective] In order to promote the development of camellia oil processing enterprises and solve the problem of waste of camellia oil enterprises, Camallia oleifera cake that tea saponin has been removed was composted to form organic fertilizer. [Method] The D-optimal mixture design method was utilized and the seed germination rate was defined as evaluation index. The optimized formula was obtained by establishing regression equation and response surface optimization analysis based on single factor experiments. [Results] Under the condition that fermentation bacteria agent was 1% of the mixture, the best composting process was: 74.8% of remove tea saponin of Camallia oleifera cake (dry), 12.5% of pig manure (dry), 8.0% of straw and 4.7% of rice husk. Its seed germination rate predicted value was 150.56%, which was basically consistent with the measured value 151.23%; Its total nutrient content was 5.17%, which was up to organic fertilizer standard (NY525-2012). [Conclusion] The optimized formula can be used as composting process of Camallia oleifera cake, which has good repeatability, and provide scientific basis for rational utilization of Camallia oleifera cake and development of organic fertilizer.

1. Introduction
Oil-tea cake is the residue of oil extracted from oil-tea camellia seed kernel, also known as tea cake, tea cake, etc. [1], with a yield of about 1.3-1.4 million tons/year[2-3], fresh products containing about 5-7% of residual oil, about 12-16% of crude protein, about 40-50% of total sugar, about 10-13% of tea saponin, etc. [4-5]. Due to the pungent taste of tea saponin, its poor palatability cannot meet the needs of higher vertebrate feed [6]. Tea saponin is a kind of pentacyclic triterpenoid saponin, which has a variety of biological activities such as antibacterial, insecticidal, molluscicidal, hemolytic, anti-tumor and so on [7-10]. It can be used as a raw material for producing bactericides, pesticides, health care products and the like, and the market price is relatively high, and the price of the tea saponin with the content of 60% is as high as 500 million yuan/ton.
Oil tea cake is usually used as the raw material for extracting tea saponin. The oil tea cake from which tea saponin has been removed is almost discarded or used as fuel, causing serious waste of resources. The content of crude protein, total sugar and nitrogen-free extract in the tea cake with tea saponin removed oil is relatively high, which has the potential to generate organic fertilizer. However, if it is directly used as fertilizer, it will also affect the fertility due to insufficient nutrients.

In recent years, the utilization of oil tea cake mainly focuses on the extraction, separation and purification of polysaccharide, protein and tea saponin [11-14], while the further utilization of oil tea cake from which tea saponin has been removed is less. Currently, only a small amount of oil tea cake is used as feed and activated carbon raw materials [15-16], and the research on organic fertilizer generated from compost is even less. Using D-optimal mixing design to optimize the mixing process has been favored by many scholars. Zhang Dong et al [17] used D-optimal mixture design to optimize the low-salt formula of cured meat. The optimized formula was 11% calcium ascorbate, 67%NaCl and 22%KCl. The taste, color and comprehensive evaluation score of the optimized low-salt cured meat reached the predicted values. Yang Hui et al [18] used chestnut as the main material and rice, wheat kernel and corn as the auxiliary materials to optimize the mixing design. The optimized formula was: 63.6% chestnut, 12.3% rice, 11.4% wheat kernel and 12.7% corn. The formula could produce reducing sugar 139.21g/L, which was consistent with the predicted value. In this study, the tea saponin-removed oil tea cake was used as the main material for aerobic composting, and D-optimal mixing design was used to optimize the composting process, so as to provide a new way for fermentation of tea saponin-removed oil tea cake residue as organic fertilizer, solve the problem of tea oil enterprise waste, and promote the development of tea seed oil processing enterprises.

2. Materials and Methods

2.1. Materials and reagents
Camellia oil cake, purchased from Guizhou Shiqian Fuding mountain wild camellia oil industry Co., Ltd., has been desaponin camellia oil cake (self-made); Pig manure, rice straw, rice husk, fermentation fungus (effective active bacteria ≥50 million / g), fermentation compost bucket, and cabbage seeds were all purchased from Sanqiao Wholesale Market in Guiyang. Main reagents: concentrated sulfuric acid, absolute ethanol, sodium hydroxide, potassium dichromate, etc. are of analytical grade.

2.2. Instrumentation and equipments
XFB-500 high-speed traditional Chinese medicine pulverizer, Jishou zhongxiang pharmaceutical machinery factory; PHS-3E pH Meter, Shanghai Leimagnetic Instrument Factory; AL204 -IC Electronic Balance, Swiss mettler toledo Company; Agilent 7500 a Inductively Coupled Plasma Mass Spectrometer, Agilent Technologies, USA; Milli-Q Synthesis ultra-pure water system, MILLIPORE company, usa; SKD-100 Automatic Kjeldahl Nitrogen Determination Instrument, Shanghai Peiou Analytical Instrument Co., Ltd.

2.3. Experimental method

2.3.1. Experimental design. The tea cake with tea saponin oil removed was mixed with auxiliary materials pig manure, straw and rice husk respectively to form a mixture test. The single factor test was carried out with the seed germination rate as an index under the conditions of different mass fractions of each mixture as factors and 1% of fermentation microbial inoculum as the mixture. The test process is as follows: (1) 15% of pig manure, 6% of straw, 3% of rice husk are fixed, and the mass fraction gradient of tea cake with tea saponin oil removed is 85%, 80%, 75%, 70%, 65% and 60%; (2) fixing 75% of tea saponin-removed oil tea cake meal, 15% of pig manure, 3% of rice husk, and the mass fraction gradient of straw is 0%, 2%, 4%, 6%, 8% and 10%; (3) fixing 75% of tea saponin-removed oil tea cake meal, 15% of pig manure, 6% of straw and 0%, 2%, 4%, 6%, 8% and 10% of rice husk mass fraction gradient; (4) fixing 75% of tea saponin-removed oil tea cake meal, 15% of pig...
manure, 6% of straw and 3% of rice husk, wherein the mass fraction gradient of pig manure is 4%, 7%, 10%, 13%, 16% and 20%; To determine the value range of each mixture, see table 1. Then, on the basis of single factor test, regression analysis was carried out using D-optimal in Design-expert software. The combination formula of the four is shown in Table 2, where A+B+C+D=100%, and the optimal composting process was obtained with the seed germination rate as the response value.

Table 1. Mixing experimental factors and levels

| Numbering | Ingredients          | Minimum value | Highest value |
|-----------|----------------------|---------------|---------------|
| A         | Pig manure           | 7%            | 16%           |
| B         | Straw                | 0%            | 8%            |
| C         | Rice hull            | 0%            | 8%            |
| D         | Dehydrated tea saponin Oil tea cake | 70% | 80% |

Table 2. Arrangement and results of mixture design experiment

| Serial number | A   | B   | C   | D   | Experimental value | Predicted value |
|---------------|-----|-----|-----|-----|-------------------|-----------------|
| 1             | 12.0| 0.0 | 8.0 | 80.0| 145.25           | 145.24          |
| 2             | 15.8| 5.1 | 4.9 | 74.2| 159.36           | 159.61          |
| 3             | 8.8 | 8.0 | 3.2 | 80.0| 147.87           | 148.06          |
| 4             | 8.8 | 8.0 | 3.2 | 80.0| 148.27           | 148.06          |
| 5             | 16.0| 0.5 | 3.5 | 80.0| 143.47           | 143.52          |
| 6             | 12.8| 4.7 | 5.1 | 77.4| 156.85           | 152.83          |
| 7             | 15.3| 7.6 | 7.1 | 70.0| 153.23           | 152.71          |
| 8             | 15.3| 7.6 | 7.1 | 70.0| 152.23           | 152.71          |
| 9             | 16.0| 4.1 | 2.4 | 77.5| 157.65           | 157.45          |
| 10            | 15.8| 0.0 | 6.9 | 77.3| 148.31           | 148.33          |
| 11            | 16.0| 8.0 | 1.8 | 74.2| 154.31           | 154.28          |
| 12            | 9.7 | 8.0 | 8.0 | 74.3| 147.26           | 147.25          |
| 13            | 12.8| 4.7 | 5.1 | 77.4| 151.28           | 152.83          |
| 14            | 12.8| 4.7 | 5.1 | 77.4| 150.45           | 152.83          |
| 15            | 12.5| 8.0 | 4.7 | 74.8| 150.53           | 150.56          |
| 16            | 16.0| 2.2 | 8.0 | 73.8| 148.12           | 148.06          |
| 17            | 12.9| 5.1 | 8.0 | 74.0| 149.58           | 149.61          |
| 18            | 14.1| 5.9 | 0.0 | 80.0| 146.83           | 147.41          |
| 19            | 7    | 5.0 | 8.0 | 80.0| 145.86           | 145.88          |
| 20            | 14.1| 5.9 | 0.0 | 80.0| 147.91           | 147.41          |

2.3.2. Composting method. The desaponin oil tea cake was crushed to the particle size ≤ 1.2cm, the pig manure to the particle size ≤ 1.0cm, the straw was cut to 1-2cm with scissors, the components were evenly added to the fermentation compost barrel according to the mixture design method, and the fermentation bacterial agent with the dry weight of the mixture of 1% was added. Ventilation is carried out by manually turning the pile once a day, the water content of the pile body is 100%-120%, and the pH value is 6.5-8.0. The thermometer is inserted into the middle of the stack in the barrel to measure the temperature. When the stack temperature approaches room temperature and does not rise any more, the composting is completed, and the total composting time is 25-30 days.

2.3.3. Rate of emergence. With reference to the germination rate determination method of Li Hongxia[19], it is slightly modified: the compost product and deionized water are mixed evenly according to the mass ratio of 1: 10, shaken on a horizontal shaker for 2 hours, centrifuged (4000 rpm) for 10 min, and left standing to take the filtrate for later use. Add 5ml of filtrate into a dish padded
with 9cm filter paper at the bottom, put 20 cabbage seeds with full seeds and the same size into each dish, put them into a 25 ± 2 ℃ incubator, cultivate them in dark for 3 days, measure their seed germination rate, and take deionized water as the control.

Seed germination percentage (%)=(germination rate of filtrate × average root length)/(germination rate of pure water × average root length)×100%

2.3.4. Determination of physical and chemical indexes. pH value of compost products is determined by potentiometric method. The organic matter value was determined by potassium dichromate heating method. Total nitrogen was determined by Kjeldahl method. Quick-acting phosphorus and quick-acting potassium were extracted by sodium bicarbonate and ammonium acetate respectively, and then determined by inductively coupled plasma atomic emission spectrometry.

3. Results and Analysis

3.1. Single factor test results

3.1.1. Effect of oil tea cake mass fraction on seed germination rate. As can be seen from fig. 1, with the increase of the mass fraction of the oil-tea cake, the seed germination rate decreases successively. Analysis of the reason may be due to the increase in the mass fraction of oil-tea cake meal, the corresponding proportion of other materials will decrease, and the total nutrient value will decrease, resulting in the decline of germination rate. Therefore, 70%-80% is selected as the optimal value range of the oil-tea cake, considering the use of the oil-tea cake as much as possible.

![Figure 1. Effect of mass fraction of oil-tea-cake husk on seed germination rate](image-url)
3.1.2. Effect of straw mass fraction on seed germination rate.

![Figure 2. Effect of mass fraction of straw on seed germination rate](image2)

As can be seen from fig. 2, with the increase of straw mass fraction, seed germination first increases and then decreases. Analysis of the reasons may be due to a small amount of straw added to increase its porosity, which is conducive to aerobic composting; However, with the increase of mass fraction, the corresponding proportion of other materials will decrease, and the total nutrient value will decrease, resulting in the decline of germination rate. Therefore, 0%-8% is selected as the optimal value range of straw.

3.1.3. Effect of rice husk mass fraction on seed germination rate.

![Figure 3. Effect of mass fraction of rice husk on seed germination rate](image3)

As can be seen from fig. 3, with the increase of rice husk mass fraction, seed germination first increases and then decreases. Analysis of the reason may be due to the small amount of rice husk added, increasing its porosity, which is conducive to aerobic composting; However, with the increase of mass fraction, the corresponding proportion of other materials will decrease, and the total nutrient
value will decrease, resulting in the decline of germination rate. Therefore, 0%-8% is selected as the optimal value range of rice husk.

3.1.4. Effect of pig manure mass fraction on seed germination rate. As can be seen from fig. 4, with the increase of pig manure mass fraction, the seed germination rate increases in turn. The reason may be due to the increase of pig manure mass fraction and its total nutrient value, resulting in the increase of germination rate. Therefore, 7%-16% is selected as the optimal value range of the oil-tea cake, considering the use of the oil-tea cake as much as possible.

![Figure 4. Effect of mass fraction of pig manure on seed germination rate](image)

3.2. D-optimal mixture design optimization test

3.2.1. Establishment of model and regression equation. Model Quadratic and Special Cubic regression equations are used for analysis. The effects of various mixing factors on seed germination rate can be expressed by the following multiple regression equations:

\[ Y = +140.79A + 136.52B + 151.53D + 32.84AB + 24.13AC + 5.54AD + 17.35BC + 15.05BD + 25.630CD - 31.95ABC - 3.05ABD - 46.63ACD + 253.86BCD \]

The germination rate \( Y \) of seeds is the response value, and \( A, B, C \) and \( D \) respectively represent the mass fractions of pig manure, straw, rice husk and tea cake with tea saponin oil removed. It can be seen from the regression equation that the absolute value of the regression coefficient of the third term is \( BCD > ACD > ABC > ABD \), which shows that the interaction of straw, rice husk and the removed tea saponin oil tea cake has a significant effect on the seed germination rate.

3.2.2. Variance analysis. Statistical analysis was carried out on the seed germination rate of compost products. The analysis results are shown in Table 2. Table 3 shows the regression and variance analysis results of seed germination rate of compost products. It can be seen from table 3 that taking the seed germination rate as the response value, the model \( P = 0.0202 < 0.0500 \), indicating that the model is significant, and the mismatch \( P = 0.8834 > 0.1000 \), indicating that the mathematical model fits well with the test results and can be used to speculate the test results. The correlation coefficient of multivariate equation is \( R^2 = 0.9264 \), which indicates that 92.64% of the data can be explained by this equation. The correction determination coefficient \( R^2_{adj} = 0.7668 \) indicates that the mathematical model can better predict the relationship between the mixing ratio and the seed germination rate. The coefficient of variation is 1.37%, which indicates that the model has high confidence and can better reflect the true value of the test, and can also be used to analyze the changes of seed germination rate of the model.
Table 3. Analysis of variance of germination rate

| Source of variance | Sum of squares | Variance | Mean square | F value | P value | Salienty |
|--------------------|----------------|----------|-------------|---------|---------|----------|
| Fitting model      | 320.95         | 13       | 24.69       | 5.81    | 0.0202  | Significant |
| Mixed linear model | 125.65         | 3        | 41.88       | 9.85    | 0.0098  |           |
| AB                 | 2.47           | 1        | 2.47        | 0.58    | 0.4751  |           |
| AC                 | 1.67           | 1        | 1.67        | 0.39    | 0.5537  |           |
| AD                 | 0.22           | 1        | 0.22        | 0.051   | 0.8289  |           |
| BC                 | 0.67           | 1        | 0.67        | 0.16    | 0.7058  |           |
| BD                 | 1.31           | 1        | 1.31        | 0.31    | 0.5984  |           |
| CD                 | 3.28           | 1        | 3.28        | 0.77    | 0.4134  |           |
| ABC                | 0.32           | 1        | 0.32        | 0.075   | 0.7932  |           |
| ABD                | 0.002          | 1        | 0.002       | 0.0006  | 0.9815  |           |
| ACD                | 0.74           | 1        | 0.74        | 0.17    | 0.6911  |           |
| BCD                | 26.76          | 1        | 26.76       | 6.29    | 0.0460  |           |
| Residual           | 25.51          | 6        | 4.25        |         |         |           |
| Missing items      | 0.12           | 1        | 0.12        | 0.024   | 0.8834  | Not significant |
| Pure difference    | 25.39          | 5        | 5.08        |         |         |           |
| Total variation    | 346.46         | 19       |             |         |         |           |

3.2.3. Response surface analysis of interaction factors. According to the experimental results and regression equations of the optimal mixture design, the contour map and effect surface map of the effects of various mixture factors on seed germination rate can be drawn. In the experiment, the contour map and effect surface map of the interaction factors of pig manure, straw, rice husk and tea cake cake with tea saponin removed oil on seed germination rate are shown in fig. 5 and fig. 6. The effect surface in fig. 5 is a curved surface with a large slope, which shows that the interaction between the mixing factors is obvious. When the mass fraction of the mixing factors is appropriate, the seed germination rate has the maximum value. When the mass fraction of tea saponin-removed oil and tea cake is 76.583%, the curved surface shows an upward trend when the mass fraction of fixed straw and rice husk and the mass fraction of pig manure increase from 7.417% to 16.0%. Fixed pig manure and rice husk mass fraction, with the increase of straw mass fraction, curved surface showed a trend of rising first and then falling. The trend of rice husk and straw is basically the same.

Figure 5. Contour map and 3D effect surface map of the effect of interaction factors on seed germination rate

3.2.4. Process optimization and verification test. According to the experimental results of the optimal mixture design and the regression equation, the optimal process is as follows: pig manure 12.5%,
straw 8.0%, rice husk 4.7% and tea cake 74.8% with tea saponin oil removed. Under this process, the model predicted the germination rate of seeds to be 150.56%. According to the mixture design method, a 3-component optimization process can be obtained to carry out validation tests, and the results are shown in Table 4. From Table 4, it can be seen that the verification test results are basically consistent with the predicted values, indicating that the obtained optimized process has high credibility with the predicted values and can be used as the composting process of oil tea cake.

Table 4. Optimized of formula verification test results

| Process | Factor/% | Seed germination rate/ (%) |
|---------|---------|---------------------------|
|         | A       | B   | C       | D       | Predicted value | Experimental value |
| 1       | 12.9    | 5.1 | 8.0     | 74.0    | 149.61          | 149.47             |
| 2       | 12.5    | 8.0 | 4.7     | 74.8    | 150.56          | 151.23             |
| 3       | 9.6     | 7.4 | 3.4     | 79.6    | 148.47          | 148.23             |

3.3. Nutrient content of compost products
The nutrient content of the oil tea cake compost product is shown in Table 5. CK represents the natural fermentation product of the tea saponin-removed oil tea cake, and Z represents the tea saponin-removed oil tea cake compost product. The total nutrient content of nitrogen, phosphorus and potassium (N, P₂O₅ and K₂O) in Z is 5.17%, which meets the organic fertilizer standard (NY525-2012).

Table 5. Nutrient content of compost products

| Handle | pH | Organic matter | Nitrogen (N) | Phosphorus(P₂O₅) | Potassium(K₂O) | Total nutrient |
|--------|----|----------------|--------------|------------------|----------------|----------------|
| CK     | 6.81 | 65.27         | 1.82         | 1.37             | 1.14           | 4.33           |
| Z      | 6.43 | 67.35         | 2.23         | 1.58             | 1.36           | 5.17           |

4. Summary
The total nutrient content of composted products was greatly improved by using tea cake with tea saponin oil removed as the main material, pig manure, straw and rice husk as the auxiliary materials and adding quantitative fermentation microbial agents, thus promoting the germination rate of seeds. The D-optimal mixture design method was used to optimize the composting process. The seed germination rate was taken as the response value, and the optimal composting process was 12.5% pig manure, 8.0% straw, 4.7% rice husk and 74.8% tea cake with tea saponin oil removed under the condition of 1% fermentation inoculum. Under this process, the model predicted the germination rate of seeds to be 150.56%. The verification test results are basically consistent with the predicted values. The optimized process of the test model has high reliability with the predicted values, and can be used as the composting process of oil tea cake. The total nutrient content of the compost product after the optimization process is 5.17%, which is greater than the national standard value of 5.0%, and meets the organic fertilizer standard (NY525-2012).

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