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Optimization of Conditions for the Extraction of Tea Seed Oil Using Cellulase and Analysis of the Tea Seed Oil

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Abstract

Tea seed oil, rich in unsaturated fatty acids, is comparable to olive oil in nutritional value and is considered a high-grade plant-based edible oil. The cellulase-assisted aqueous enzymatic method involves mechanically crushing and roasting tea seeds, followed by the addition of cellulase to degrade the cell walls, releasing the oil from the cells, and finally collecting the oil via centrifugation. This study optimized five factors affecting the extraction process: enzymatic hydrolysis time, enzyme dosage, solid-to-liquid ratio, temperature, and pH. The optimal conditions for cellulase-assisted extraction of tea seed oil were determined to be an enzymatic hydrolysis time of 75 minutes, enzyme dosage of 60 U/g of tea seed powder, solid-to-liquid ratio of 1:4, temperature of 45°C, and pH of 4.5, achieving a maximum extraction yield of approximately 18%. Additionally, the moisture and volatile content, peroxide value, and acid value of the extracted tea seed oil were analyzed. The results showed that the oleic acid content in the extracted oil was 70.49%, and the total unsaturated fatty acid content was 76.74%. Both the acid value and peroxide value were lower than the national standards for crude tea seed oil. This study provides a reference for the application of cellulase-assisted aqueous enzymatic extraction of tea seed oil.

Keywords

Cellulase; Tea Seed Oil; Aqueous Enzymatic Method; Composition Analysis.

1. Introduction

Camellia oleifera is a unique and important oil-producing tree in southern China, and tea seed oil is a high-quality edible oil extracted from its seeds [1]. Tea seed oil is rich in unsaturated fatty acids, vitamins, and minerals, with a unique flavor, high digestibility, and long storage life [2]. Its composition and physicochemical properties are similar to those of olive oil, but it is more cost-effective, making it a promising alternative in the food, pharmaceutical, and cosmetic industries [3].

Traditional methods for extracting tea seed oil include mechanical pressing and solvent extraction [3]. Mechanical pressing requires high-temperature roasting, which can lead to protein denaturation, low oil yield, high residual oil in the cake, and potential contamination with benzo[a]pyrene [4]. Solvent extraction can achieve an oil yield of up to 95% [4], but it involves high energy consumption during solvent separation and poses safety and environmental risks due to the use of flammable and toxic organic solvents [4]. With the current cultivation area of Camellia oleifera reaching 6.55 million hectares and an annual production of 500,000 to 600,000 tons of tea seed oil, the industry is expected to grow significantly, with an estimated production of 2.5 million tons by 2025 [5]. Therefore, there is an urgent need to develop a high-yield, safe, and environmentally friendly extraction method.

The aqueous enzymatic method involves mechanically crushing the plant material, adding enzymes to degrade the cell walls, releasing the oil, and then collecting the oil via centrifugation.

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Compared to traditional methods, the aqueous enzymatic method achieves a similar oil yield to solvent extraction [6], but it requires less specialized equipment, operates under mild conditions, avoids high temperatures and organic solvents, and allows for the comprehensive utilization of by-products [7]. This method has been successfully applied to extract oils from tomato seeds [8], avocado [9], Moringa seeds [10], Idesia polycarpa [11], pumpkin seeds [12], and walnuts [13], as well as tea seeds [14-16]. Among these, the extraction of tea seed oil using the aqueous enzymatic method has gained significant attention. For example, Guo Yanhong studied the optimal conditions for single-enzyme extraction of tea seed oil [17], Ji Peng investigated the effects of microwave-assisted extraction [3], and Fang Xuezhi explored the optimal conditions for multi-enzyme extraction and demulsification methods [7]. Previous studies have shown that the reaction times for amylase and alkaline protease-assisted extraction of tea seed oil are 3 hours [3] and 4 hours [4], respectively. In contrast, cellulase-assisted extraction directly degrades the cellulose in the cell walls of tea seeds, potentially reducing the reaction time and improving production efficiency. Therefore, this study focuses on the cellulase-assisted aqueous enzymatic extraction of tea seed oil.

Current research on tea seed oil has evolved from simple extraction optimization to the analysis of its composition [18, 19], volatile compounds [20, 21], and health benefits [22]. For example, Wang Xingjin et al. used GC-FID to analyze the fatty acid composition of tea seed oil to assess its quality [18], Han Xiaomiao et al. studied the volatile components of tea seed oil extracted using five different methods [20], and Li Ziming et al. investigated the effects of refining on the fatty acid and volatile compound profiles of tea seed oil [21]. Pan Chao et al. explored the effects of tea seed oil on alleviating physical fatigue [22]. Additionally, the rancidity of tea seed oil is a major concern during processing, with peroxide value and acid value being key indicators of freshness and safety [23-27]. He Zhehua et al. compared the acid value, peroxide value, and benzo[a]pyrene content of tea seed oil extracted using three different methods [23]. However, there is limited research on the composition and quality of tea seed oil extracted using the aqueous enzymatic method. This study not only optimizes the conditions for cellulase-assisted extraction but also analyzes the acid value, peroxide value, and composition of the extracted oil, providing a reference for future applications of this method.

2. Materials and Methods

2.1. Materials, Reagents, and Instruments

Tea seeds were purchased from Yunwu Mountain Tea Farm in Suizhou. Cellulase (30,000 U/g, optimal temperature 30-50°C, optimal pH 4-5) was purchased from TCI (Shanghai) Chemical Industry Co., Ltd. Sodium sulfate, ethanol, potassium iodide, petroleum ether, ether, chloroform, glacial acetic acid, and other reagents were of analytical grade, and 0.1 mol/L sodium thiosulfate standard solution was purchased from Chengdu Kelong Chemical Reagent Factory.

The instruments used included a ultrapure water system (UPH-11-21T, Chengdu Ultrapure Technology Co., Ltd.), a thermostatic magnetic stirrer (DF-101S, Yuhua Instrument Co., Ltd.), a centrifuge (TGL-16, Sichuan Shuke Instrument Co., Ltd.), a gas chromatography-mass spectrometer (TSQ 8000, Thermo Scientific), an analytical balance (ESJ182-4, Shenyang Longteng Electronic Co., Ltd.), and an oven (T1-L101B, Midea Kitchen Appliances Manufacturing Co., Ltd.).

2.2. Materials and Methods

2.2.1. Preparation of Tea Seed Oil Using the Aqueous Enzymatic Method

Shelled camellia seeds and dried at 70℃ for 4 h. Let the tea seeds cool and crush them with a grinder. After sifting 30 mesh tea seed powder, place it in refrigerator at 4℃ for later use. Weigh 21 g tea seed powder and place it in 250 mL beaker, add a certain volume of citric acid-disodium hydrogen phosphate buffer at a certain pH, and put it in a water bath to deactivate the enzyme for 21 min (80℃). After the solution has cooled to room temperature, a certain amount of cellulase is added to the beaker. Then, the water bath was heated at a certain temperature, the enzymatic hydrolysis was carried out for a certain time, and the enzyme was inactivated for a second time for 21 min (80℃) after the enzymatic hydrolysis was finished. The mixed liquid was collected, centrifuged (8000 rpm, 10 min), the upper clear oil was taken, weighed, and the extraction rate of tea seed oil was calculated according to formula (1). The extracted tea seed oil is stored in a refrigerator at 4℃. Each experiment was repeated twice.

$$R = \frac{\mathrm{m_o}}{\mathrm{m_s}} \tag{1}$$

Where $\mathrm{m_o}$ represents the mass of the upper clear oil, and while $\mathrm{m_s}$ refers the mass of tea seed powder.In formula (1), the mass of tea seed powder is 21 g.

2.2.2. Determination of Moisture and Volatile Content in Tea Seed Oil

The moisture and volatile content in the extracted tea seed oil was determined using the air oven method (GB/T 5528-2008) [28]. The experiment was repeated three times.

2.2.3. Determination of Peroxide Value in Tea Seed Oil

The peroxide value of the extracted tea seed oil was determined using the sodium thiosulfate titration method [29]. The experiment was repeated three times.

2.2.4. Determination of Acid Value in Tea Seed Oil

The acid value of the extracted tea seed oil was determined using the acid-base titration method [30]. The experiment was repeated three times.

2.2.5. Composition Analysis of Tea Seed Oil

The fatty acid composition of the extracted tea seed oil was analyzed using GC-MS [31, 32]. The injection port temperature was set at 250°C, the FID detector temperature at 270°C, and the column temperature was programmed from 180°C (5 minutes) to 240°C at a rate of 5°C/min (held for 5 minutes). The split ratio was 21:1, with hydrogen flow at 45 mL/min, air flow at 400 mL/min, nitrogen flow at 1.5 mL/min, and make-up gas flow at 30 mL/min. The experiment was repeated twice.

3. Results and Discussion

3.1. Optimization of Extraction Conditions

3.1.1. Effect of Enzymatic Hydrolysis Time on Extraction Yield

The effect of enzymatic hydrolysis time on extraction yield is shown in Figure 1.

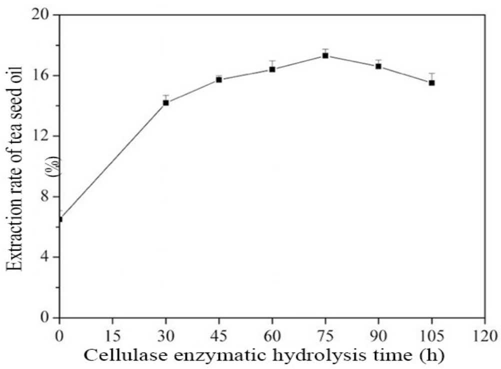

Fig. 1 Effect of enzymatic hydrolysis time on extraction yield.

Fig 1. Effect of hydrolysis time of cellulase on extraction yield: 21 g tea seed powder, solid-liquid ratio 1:4 (80 mL pH 4.5 citric acid-disodium hydrogen phosphate buffer), 0.038 g (60 U/ tea seed powder g) cellulase, hydrolysis temperature 45℃. The enzymolysis time was 0 min, 45 min, 60 min, 75 min, 90 min, 105 min.

The results show that the extraction yield was highest at 75 minutes of enzymatic hydrolysis. As the hydrolysis time increased from 0 to 75 minutes, the extraction yield significantly increased. However, beyond 75 minutes, the yield gradually decreased. This is because prolonged hydrolysis increases the likelihood of fatty acids forming emulsions with water, making phase separation difficult and reducing the oil yield [33]. Additionally, prolonged hydrolysis may lead to the dissolution of non-lipid components, complicating oil collection [34]. Therefore, 75 minutes was selected as the optimal hydrolysis time.

3.1.2. Effect of Enzyme Dosage on Extraction Yield

The effect of enzyme dosage on extraction yield is shown in Figure 2.

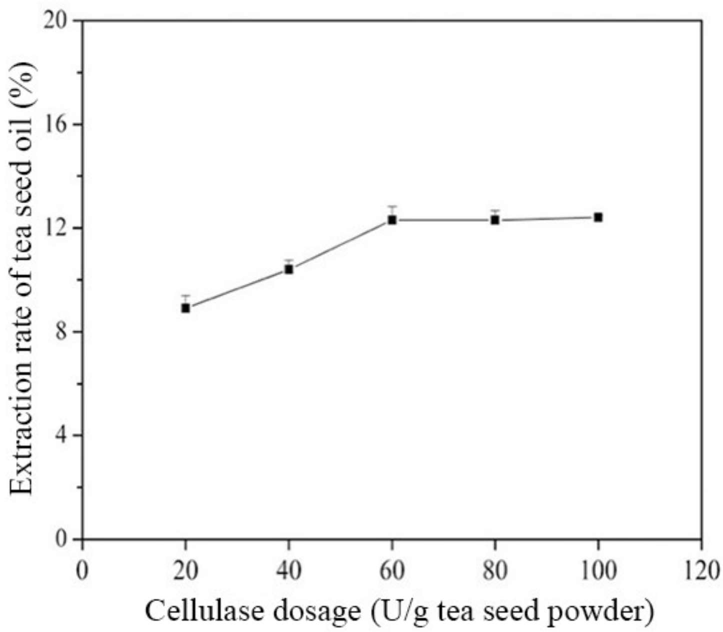

Fig. 2 Effect of enzyme dosage on extraction yield.

Fig 2. Effect of cellulase amount on extraction yield: 21 g tea seed powder, solid-liquid ratio 1: 4 (80 mL pH 4.5 citric acid-disodium hydrogen phosphate buffer), adding cellulase 0.013 g (21 U/ tea seed powder g), 0.025g (40 U/ tea seed g), 0.038g (60 U/ tea seed g), 0.051g (80 U/ tea seed g), 0.063g (100 U/ tea seed g). The enzymolysis temperature was 45 ℃ and the enzymolysis time was 75 min.

The results show that the extraction yield was highest at an enzyme dosage of 60 U/g of tea seed powder. As the enzyme dosage increased from 21 to 60 U/g, the extraction yield increased. However, beyond 60 U/g, the yield plateaued, indicating that the amount of tea seed powder became the limiting factor [33]. Therefore, 60 U/g was selected as the optimal enzyme dosage.

3.1.3. Effect of Solid-to-Liquid Ratio on Extraction Yield

The effect of solid-to-liquid ratio on extraction yield is shown in Figure 3.

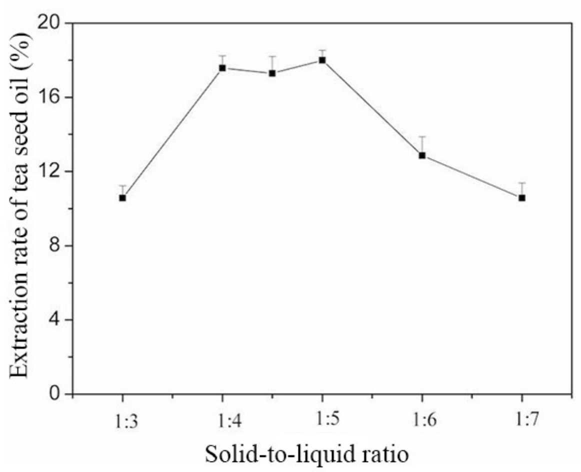

Fig. 3 Effect of solid-to-liquid ratio on extraction yield.

Fig 3. Effect of cellulase solid-liquid ratio on extraction rate: 21 g tea seed powder, adding 60 mL (solid-liquid ratio 1:3), 80 mL (solid-liquid ratio 1:4), 90 mL (solid-liquid ratio 1:4.5), 100 mL (solid-liquid ratio 1:5), 121 mL (solid-liquid ratio 1:6), 140 mL (solid-liquid ratio 1:7) pH 4.5 Citric acid-disodium hydrogen phosphate buffer, Add cellulase 0.038 g (60 U/ tea seed powder g). The enzymolysis temperature was 45℃ and the enzymolysis time was 75 min.

The results show that the extraction yield was highest at a solid-to-liquid ratio of 1:4. As the ratio increased from 1:3 to 1:4, the extraction yield increased. However, beyond 1:4, the yield decreased due to reduced enzyme and substrate concentrations, leading to lower extraction efficiency [33]. Additionally, higher solvent volumes may promote the formation of emulsions, further reducing the yield [34]. Therefore, a solid-to-liquid ratio of 1:4 was selected as optimal.

3.1.4. Effect of Temperature on Extraction Yield

The effect of temperature on extraction yield is shown in Figure 4.

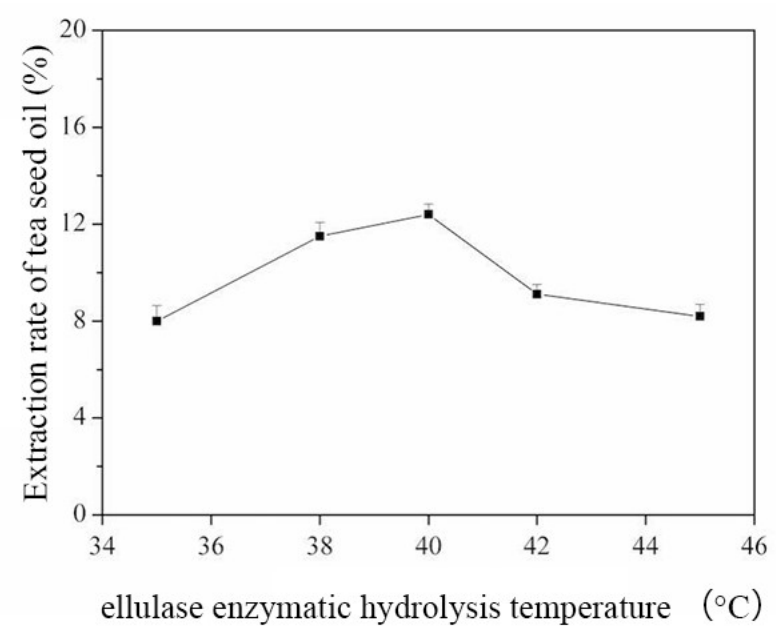

Fig. 4 Effect of temperature on extraction yield.

Fig. 4. Effect of cellulase enzymatic hydrolysis temperature on extraction rate: 21 g of tea seed powder, 80 mL (solid-to-liquid ratio 1:4) of pH 4.5 citrate-phosphate buffer, and cellulase dosage of 0.038 g (60 U/g tea seed powder). Enzymatic hydrolysis temperatures were 41°C, 42°C, 45°C, 47°C, and 49°C, with a hydrolysis time of 75 min.

The results show that the extraction yield was highest at 40°C. As the temperature increased from 36°C to 40°C, the extraction yield increased due to enhanced enzyme activity and faster diffusion of oil molecules [34]. However, beyond 40°C, the yield decreased due to enzyme denaturation [36]. Therefore, 40°C was selected as the optimal temperature.

3.1.5. Effect of pH on Extraction Yield

The effect of pH on extraction yield is shown in Figure 5.

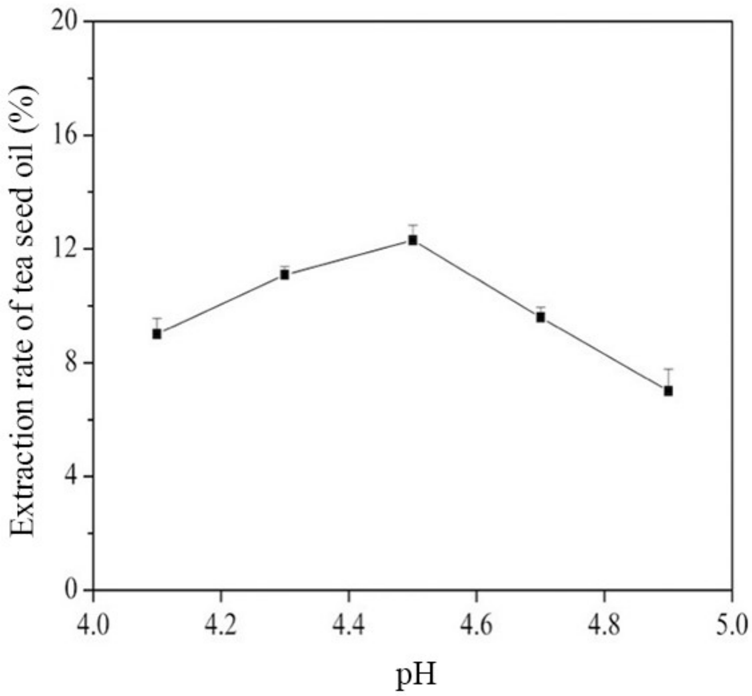

Fig. 5 Effect of pH on extraction yield.

Fig. 5 shows the effect of cellulase reaction temperature on the extraction rate: 21 g tea seed powder was added with 80 mL (solid-liquid ratio 1:4) citric acid-disodium hydrogen phosphate buffer at pH 4.1, 4.3, 4.5, 4.7, 4.9, cellulase 0.038 g (60 U/ tea seed powder g). The enzymolysis temperature was 45℃ and the enzymolysis time was 75 min.

The results show that the extraction yield was highest at pH 4.5. As the pH increased from 4.1 to 4.5, the extraction yield increased due to enhanced enzyme activity. However, beyond pH 4.5, the yield decreased due to reduced enzyme activity [37]. Therefore, pH 4.5 was selected as optimal.

3.2. Quality of Tea Seed Oil Extracted Using Cellulase

The quality of the extracted tea seed oil was evaluated based on moisture and volatile content, peroxide value, and acid value, as shown in Table 1.

The results show that the peroxide value and acid value of the extracted oil were lower than the national standards (peroxide value ≤ 7.5 mmol/kg, acid value ≤ 4.0 mg/g, GB/T 11765-2018 [38]), indicating that the oil was fresh and of high quality. However, the moisture and volatile

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content exceeded the national standard (≤ 0.2%), suggesting that further drying is needed to improve storage stability and flavor.

Table 1. Moisture and volatile content, peroxide value, and acid value of tea seed oil.

Tea Seed Oil	Moisture and Volatile Content (%)	Peroxide Value (mmol/kg)	Acid Value (mg/g)
Value	0.66	2.34	0.71

3.3. Effect of Enzyme Type on Tea Seed Oil Composition

The GC chromatogram of tea seed oil extracted using cellulase is shown in Fig. 6.

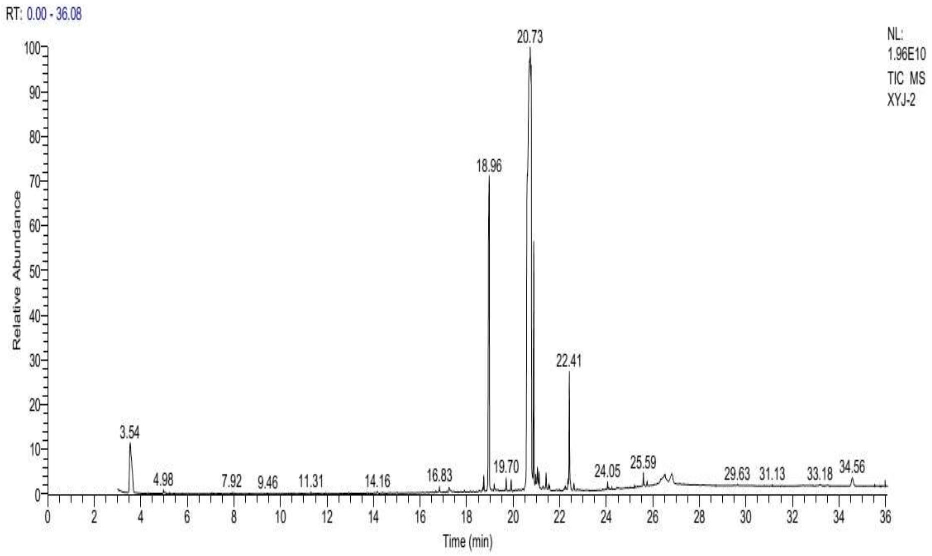

Fig. 6 GC spectrum of tea seed oil extraction by cellulase water enzymatic method.

The relative content of each fatty acid in the extracted oil was determined using peak area normalization [18, 39], as shown in Table 2.

Table 2. Composition of tea seed oil.

RT (min)	Fatty Acid Name	Molecular Formula	Relative Content (%)
16.83	Myristic Acid	$C_{14}H_{28}O_2$	0.18
18.73	Palmitoleic Acid	$C_{16}H_{30}O_2$	0.48
18.96	Palmitic Acid	$C_{16}H_{32}O_2$	14.41
20.73	Oleic Acid	$C_{18}H_{34}O_2$	70.49
20.88	Stearic Acid	$C_{18}H_{36}O_2$	5.91
21.04	Linoleic Acid	$C_{18}H_{32}O_2$	1.44
21.41	Arachidonic Acid	$C_{20}H_{38}O_2$	0.47
22.41	Eicosenoic Acid	$C_{20}H_{38}O_2$	0.18

The results show that the extracted oil contained six unsaturated fatty acids, with oleic acid being the most abundant at 70.49%. The total unsaturated fatty acid content was 76.74%,

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indicating that the oil has significant health benefits, such as regulating blood lipids, lowering blood sugar, and protecting heart health [40].

4. Conclusion

The optimal conditions for cellulase-assisted aqueous enzymatic extraction of tea seed oil are: enzymatic hydrolysis time of 75 minutes, enzyme dosage of 60 U/g of tea seed powder, temperature of 45°C, solid-to-liquid ratio of 1:4, and pH of 4.5 (citrate-phosphate buffer). Under these conditions, the maximum extraction yield was approximately 18%. This method requires a relatively low enzyme dosage and achieves a high extraction yield.

The extracted tea seed oil had an oleic acid content of 70.49% and a total unsaturated fatty acid content of 76.74%. Both the acid value and peroxide value were lower than the national standards for crude tea seed oil, indicating high-quality oil. However, the moisture and volatile content exceeded the standard, suggesting the need for further drying.

Although cellulase is relatively expensive, this method offers a high extraction yield and produces high-quality oil. Future research should focus on enzyme recycling and by-product utilization to reduce costs.

Acknowledgments

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