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Research Article

Optimum Extraction Technology for the Seed Oil of *Nigella sativa* L.

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Seed oil of *Nigella sativa* L. is a popular edible oil in Egypt. How to improve the extraction efficiency of the oil will expand the use of the resource. In this paper, the four extraction factors, particle size of the seed, liquid-seed ratio, extraction time, and temperature, were optimized by the single-factor and orthogonal experiment to increase the extraction yield and linoleic acid concentration. The results showed that the twice extraction technology could increase the oil rate of 23.55% compared with the once extraction technology. The extraction rate of the once extraction could reach 33.51% under the conditions of liquid-seed ratio: 9 : 1 mL/g, extraction temperature: 35°C, extraction time: 1 h, and particle size: 60 meshes. The optimum twice extraction conditions with the extraction rate of 43.78% were 8 : 1 ml/g, 40°C, 1.5 h, and 60 meshes. Besides, the highest concentration of linoleic acid (58.09 mg/g) was twice extraction condition with 7 : 1 mL/g, 40°C, 2 h, and 60 meshes.

1. Introduction

Nigella sativa L. (Ranunculaceae), a native plant in North Africa, Southern Europe, and Southwest Asia [1–4], is commonly known as cooking oil used for the curry, cake, Mediterranean cheese, and medicinal purposes in Egypt and Greece for thousand years [5]. According to the previous researches, many biological activities of the seed oil are discovered, such as irritating the digestive system, expelling wind, relieving pain, diuretic effect, sweating, antiworm, anti-inflammatory, treating asthma, tonifying kidney, invigorating brain, strengthening spleen, antioxidant, and so on [6–10]. Recently, some new functions of the seed oil, antitumor, antidiabetic, anticholesterol, and antiepilepsy are reported [2, 11–15]. In the seed, linoleic acid as unsaturated fatty acids is essential to our health [16]. Nowadays, the seeds of *N. sativa* as the multifunctional food are necessary to determine an efficient extraction method in Egypt, while most researchers are focusing on the component analysis and quality stability [10, 17] instead of evaluating the extraction technology. At present, the extract methods of the edible oil include cold pressing, supercritical CO_2 extraction, microwave-assisted extraction, and solvent extraction [10, 17], and solvent extraction is popularly used in many factories with high efficiency, simple operation, and high purity [18, 19].

Therefore, we optimized the factors of extraction technology to increase the yield of oil and the concentration of linoleic acid by single-factor test and orthogonal test.

2. Materials and Method

2.1. Materials. The seeds of *N. sativa* were collected from Giza, Egypt, in October, 2017 by Dr. Adel Fahmi Ahmed (Medicinal and Aromatic Plants Researches Department, Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt).

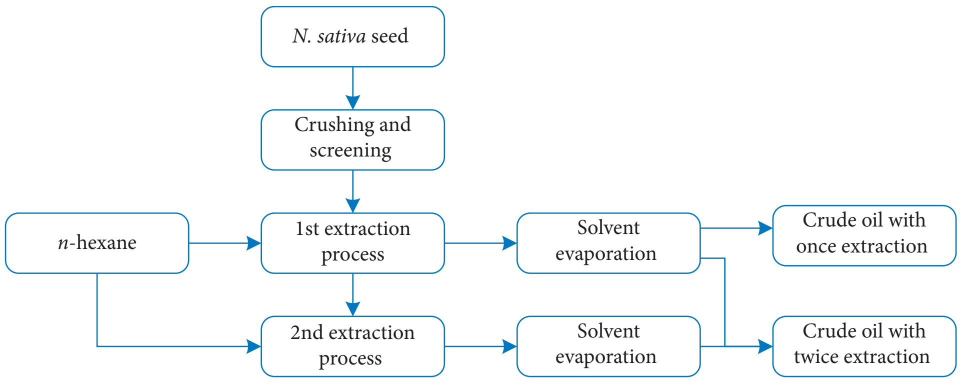

FIGURE 1: Flow chart of *N. sativa* seed oil extraction.

n-Hexane was chosen as the extraction solvent in this research [20, 21]. Linoleic acid (purity is 90.9%, batch no. 167466) was purchased from Dr. Ehrenstorfer GmbH.

The method of HPLC (high-performance liquid chromatography) was performed by using the Shimadzu LC-20AT HPLC system with an LC-20AT infusion pump, CTO-10AS column oven, SPD-20A UV detector, LC-Solution chromatographic data analysis system, and Athena C30 chromatographic column.

2.2. Extraction Technology of N. sativa Seed. According to references [22–25], the particle size of solid materials, liquid volume for extraction, and extraction temperature and time are all the key factors of oil extract technology. So, these factors were adopted to optimize extract technology of *N. sativa* seed oil in this research. Meanwhile, twice extraction method, which has been proved to be an efficiency method in the aspects of input and output [26, 27], was carried out in this work. The detailed solvent extraction processes of *N. sativa* seed are shown in Figure 1.

The oil yield can be calculated by using the following formula:

$$\text{extraction rate} = \frac{\text{weight of extracted oil}}{\text{weight of the seed}} \times 100\%. \quad (1)$$

In addition, two steps were conducted for refining the oil extract technology. In the first step, three comparatively ideal levels of the four factors were obtained by a series of single-factor tests. And then, an orthogonal test with four factors and three levels was employed to determine the optimal technology.

2.3. Determination of Linoleic Acid in N. sativa Seed Oil. The standard of linoleic acid was weighed preciously, dissolved in the methanol as mother liquor. After that, the mother liquor was diluted into a series of standard solutions with 3.80, 2.28, 1.52, 0.76, and 0.38 mg/mL, respectively. The standard solutions and samples of extracted oil were all chromatographically analyzed on a PLATINUM C30 column (4.6 mm × 250 mm, 5 μm).

According to the previous research [28], the mobile phase was a mixture of acetonitrile (A)-0.1% phosphoric

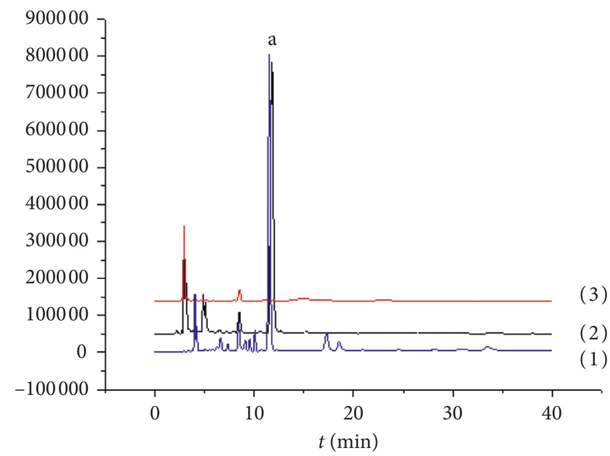

FIGURE 2: HPLC chromatograms of standard substance and *N. sativa* oil sample. (1) Sample solution; (2) standard solution; (3) solvent; (a) linoleic acid.

acid-water (B), which were used in isocratic elution at 90% A and 10% B for 40 min. The flow rate was 1.0 mL/min, and the column temperature was 30°C. The UV detection wavelength was 203 nm with the sample volume of 10 μL. The chromatograms are shown in Figure 2.

In order to test the suitability of the method, repeatability, stability, precision, and recovery tests were examined based on the previous HPLC condition:

(i) Six oil samples extracted from the *N. sativa* seed were analyzed, and the relative standard deviation (RSD) was 1.18% which meant that the method had a good repeatability

(ii) One reference solution was tested 6 times in order to test the precision and a desired result (RSD = 0.81%) was achieved

(iii) In order to test the stability of the method over time, one oil sample was detected at 0, 4, 8, 12, 16, 20, and 24 h, respectively, and the result showed that the examine result (RSD = 2.68%) would remain stable at least 24 h

(iv) Six oil samples with 80% more standard linoleic acid were tested, and the average rate of recovery and RSD were 98.70% and 1.13%, respectively

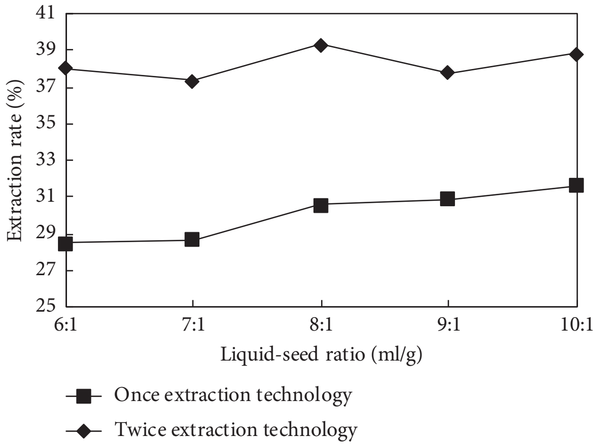

Figure 3: Effect of liquid-seed ratio on the yield of *N. sativa* seed oil.

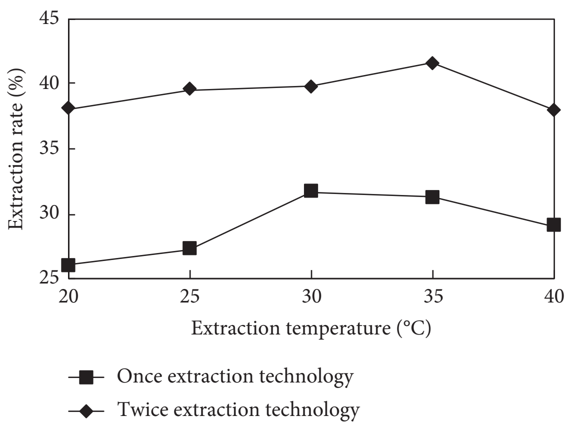

Figure 4: Effect of extraction temperature on the yield of *N. sativa* seed oil.

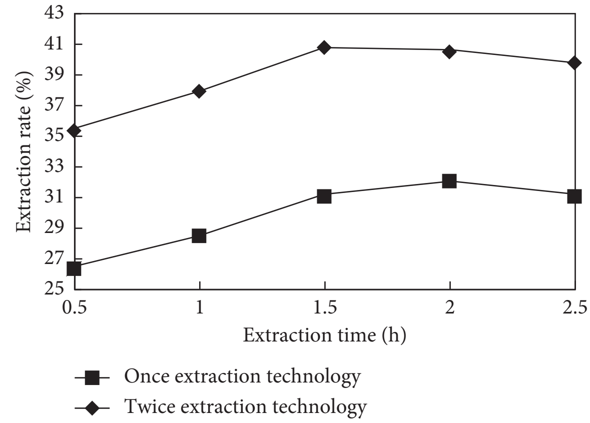

Figure 5: Effect of extraction duration on the yield of *N. sativa* seed oil.

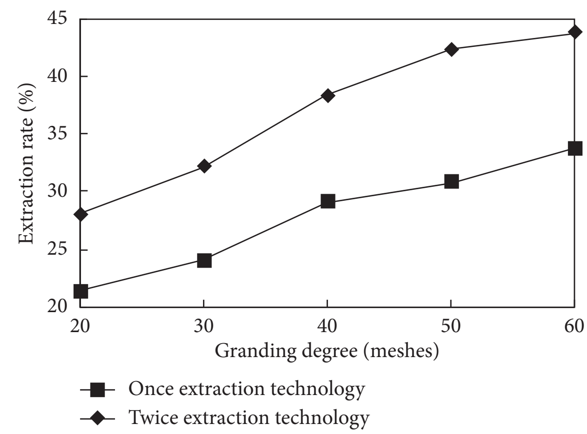

Figure 6: Effect of the grinding degree on oil yield of *N. sativa* seed oil.

According to the evaluation of the method, the results indicated that the HPLC condition was qualified for the analysis of linoleic acid in the oil.

The standard curve for analysis of linoleic acid was obtained in the equation, $Y = 9366842X - 1156864$ ($r = 0.9996$), where Y is the peak area and X is the concentration of standard with the linear range of 0.38~3.80 mg.

3. Single-Factor Analysis of the Extraction Technology of *N. sativa* Seed Oil

3.1. Effect of the Liquid-Seed Ratio on the Extraction Rate. As shown in Figure 3, the oil yield could increase with the increase of solvent volume. When the ratio of liquid and seed was more than 8 : 1 mL/g, the extraction rate of the first extraction step increased slower than before, while the oil yield of the twice extraction started to fluctuate. It is well known that the increase of the solvent could accelerate the diffusion of oil from seed, and it is an effective measure to increase the oil yield in the previous stage. But the growing trend with the increasing solvent would become less and less when the ratio is high enough, especially for the twice extraction technology [27]. So, the ratio range of the liquid and seed was determined as 7 : 1, 8 : 1, and 9 : 1 mL/g.

3.2. Effect of the Extraction Temperature on the Extraction Rate. The extraction temperatures were investigated and the results are shown in Figure 4. It presented a downward trend after the first rise from the range of 20 to 40°C. The peak temperature of the first extraction technology was at 30°C while that of the twice extraction was at 35°C. Increasing temperate of the oil would decrease the viscosity and accelerate the molecule movement of oil, but the high temperature would evaporate too much solvent (*n*-hexane) [22]. That was why the extraction yield changed as shown in Figure 4. According to the trend of the yields of the two extraction processes, 30°C, 35°C, and 40°C were chosen.

3.3. Effect of the Extraction Time on the Extraction Rate. The relationship between oil yield and extraction time is shown in Figure 5. When the extraction time was extended, the yield of the oil would increase from 0.5 to 1.5 h and then decline. It might be the time-consuming diffusion process of the oil in solvent. Along with the diffusion process, the

Table 1: The factors and levels of orthogonality experiment for the *N. sativa* seed oil extract technology.

Levels	R (ml/g)	T (°C)	t (h)	M (meshes)
1	7 : 1	40	1	40
2	8 : 1	35	1.5	50
3	9 : 1	30	2	60

Table 2: Significant analysis of the oil yield in the second extraction process.

Average extraction rate of the 1st extraction step	The 2nd extraction step				
	Average extraction rate	Standard deviation	t inspection value	d*f*	$t_{0.01}$ (one side)
33.51%	7.86%	1.06%	38.53	26	2.479

solvent would evaporate or most solvent and oil would be trapped in oil residue [18, 29]. So, the yields of the once and twice extraction technologies would decrease at 2 h and 1.5 h extraction, respectively.

3.4. Effect of the Grinding Degree on the Extraction Rate. In Figure 6, the mesh number of the seed was an important factor which had a remarkable positive relationship with the yield of the extraction oil. Smaller size of particle caused by stronger grinding was good at increasing the surface of the solid material. Besides, the grinding process can destroy the cell structure of the seed and reduce the transportation resistance of oil. [30] The increasing speed of the oil yield for the twice extraction decreased when the mesh number of the seed was over 50. In aspect of the once extraction technology, the increasing speed decreased when the grinding degree of seed reached 40 meshes. So, the mesh number was determined to be around 50 meshes.

4. Results and Discussion

4.1. Design of the Orthogonality Experiment. Based on the result of the single-factor analysis, three levels of the liquid-seed ratio (R), extraction temperature (T), extraction time (t), and grinding degrees (M) were considered in the L9(3^4) orthogonal test. The concentration of linoleic acid of the twice extraction technology was considered as one critical parameter to evaluate the twice extraction technologies besides the extraction rate of the seed oil. The detailed arrangements of the orthogonal test are listed in Table 1.

4.2. Effect of the Extraction Time on the Extraction Ratio. In order to compare the different effect of the two extraction technologies on the total yield, statistical analysis of oil production was conducted and the results are shown in Table 2.

The test result showed that the second extraction process could increase the extraction rate by 7.86% significantly through the t value test. Although the twice extraction technology would cost twice of the solvent, time, and labor, the second extraction process could improve almost one quarter of the extraction yield (almost 23.46% of the average extraction rate of the 1st extraction step) and decrease the waste of the oil significantly indeed. Therefore, it was largely necessary to implement the twice extraction technology.

4.3. Range Analysis of the Orthogonal Test. The $\mathrm{K}i_1$ and $\mathrm{K}i_2$ were the average extraction rates of the once and twice extraction technology with i level, respectively, and $i = 1$, 2, or 3. $\mathrm{K}_{\mathrm{linoleic}}$ is the average concentration of the linoleic acid in the twice extraction oil, and R_1, R_2, and $\mathrm{R}_{\mathrm{linoleic}}$ are the corresponding ranges.

In Table 3, the order of factors to affect the extraction efficiency of the first extraction process from strong to weak is grinding degree, extraction temperature, liquid-seed ratio, and extraction time, respectively, while that of the twice extraction technology was grinding degree, extraction temperature, extraction time, and liquid-seed ratio. With the increasing times of the extraction process, the effect of the liquid-seed ratio and extraction time declined obviously, especially the liquid-seed ratio. For the linoleic acid concentration in the oil obtained by the twice extraction technology, grinding degrees was still the most important factor, followed by extraction time, extraction temperature, and liquid-seed ratio.

Through the range analysis of the orthogonality experiment, the corresponding production strategies are listed in Table 4. Though the ideal conditions for the total yield and linoleic acid concentration of the twice extraction technology were different, the extraction condition with the highest yield also extract the largest amount of the linoleic acid.

4.4. Variance Analysis of the Orthogonal Test. In order to confirm the importance of these factors, variance analysis was conducted after the orthogonal test and the results are listed in Table 5.

The extreme significance of the total deviation and mean deviation revealed that the four factors considered in this paper could actually affect the oil yield of *N. sativa* seeds. The grinding degree and extraction temperature were the key factors with the extremely significant effect for the once extraction technology. But for the twice extraction technology, only the grinding degree was the main factor. More time, solvent, and operation during the twice extraction technology obviously declined the effect of extraction temperature and solvent volume.

Table 3: Analytical results of orthogonality experiment.

序号	R	T	t	M	Yield$_1$ (%)	Yield$_2$ (%)	Linoleic acid (mg/g)
1	1	1	1	1	31.62	39.49	33.44
2	1	2	2	2	33.19	41.86	47.92
3	1	3	3	3	34.73	42.83	58.09
4	2	1	2	3	35.33	43.78	56.24
5	2	2	3	1	31.65	38.42	43.02
6	2	3	1	2	32.69	42.03	39.97
7	3	1	3	2	35.21	42.55	38.44
8	3	2	1	3	36.47	43.47	54.00
9	3	3	2	1	30.67	37.89	41.71
$K1_1$	33.18	34.05	33.59	31.31			
$K2_1$	33.22	33.77	33.06	33.70			
$K3_1$	34.12	32.70	33.87	35.51			
$K1_2$	41.39	41.94	41.67	38.60			
$K2_2$	41.41	41.25	41.18	42.14			
$K3_2$	41.30	40.92	41.27	43.36			
$K1_{\text{linoleic}}$	46.49	42.71	42.47	39.39			
$K2_{\text{linoleic}}$	46.41	48.31	48.62	42.11			
$K3_{\text{linoleic}}$	44.72	46.59	46.52	56.11			
R_1	0.94	1.35	0.81	4.20			
R_2	0.11	1.02	0.49	4.76			
R_{linoleic}	1.77	5.61	6.15	16.72			

Table 4: The production strategies of the extraction technology based on the orthogonality experiment.

	R (ml/g)	T (°C)	t (h)	M (meshes)
Yield of once extraction technology	9 : 1	35	1	60
Yield of twice extraction technology	8 : 1	40	1.5	60
Concentration of linoleic acid	7 : 1	30	2	60

Table 5: Variance analysis of the orthogonality experiment.

	SSE_1	SSE_{Total}	df	F_1	F_{Total}	$F_{0.05}$	$F_{0.01}$
Total deviation	106.69	130.28	26	7.75^{**}	6.45^{**}	2.13	2.97
Mean deviation	97.16	116.30	8	22.93^{**}	18.71^{**}	2.51	3.71
Error deviation	9.53	13.98	18	—	—	—	—
R deviation	5.02	0.06	2	4.74^{*}	0.04	3.55	6.01
T deviation	9.23	4.89	2	8.72^{**}	3.15	3.55	6.01
t deviation	2.99	1.22	2	2.82	0.79	3.55	6.01
M deviation	79.92	110.12	2	75.44^{**}	70.87^{**}	3.55	6.01

Note. * and ** mean $P > 0.95$ and $P > 0.99$, respectively.

5. Conclusion

In this paper, the single-factor test and the orthogonal test were used to optimize the oil extraction technology from the seed of *N. sativa* harvested in Egypt. The ideal level of grinding degree, extraction temperature, liquid-seed ratio, and extraction time for the once and twice extraction technologies were 60 meshes, 35°C, 9 : 1 mL/g, and 1 h and 60 meshes, 40°C, 8 : 1, and 1.5 h, respectively. The extraction rate of the two technologies could achieve 33.51% and 41.37%. Only the grinding degree for the second extraction technology had significant effect on the extraction rate. As for the linoleic acid with the concentration of 58.04 mg/g in the oil from the twice extraction, the optimal extraction condition was 60 meshes, 40°C, 7 : 1 mL/g, and 2 h.

Data Availability

All original data used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

All authors declare that there are no conflicts of interest regarding this study.

Acknowledgments

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Supplementary Materials

The data used to support the findings of this study are provided. The first part: the data for the single-factor test. The second part: the data for the orthogonal test. (*Supplementary Materials*)

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