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# Extraction Technology, Component Analysis, and Biological Activity of Essential Oils from *Ligusticum jeholense* Nakai et Kitag. Vegetables

**Miao Wang [1], Jiangkuo Li [2,*], Yamin Xu [3], Pengyu Liu [4], Qiang Zheng [1], Xia Bai [1] and Donghua Li [1,*]**

[1] College of Environmental and Safety Engineering, Shenyang University of Chemical Technology, Shenyang 110142, China; 15141271452@163.com (M.W.); zhengq_neu@163.com (Q.Z.); 15511025506@139.com (X.B.)
[2] National Engineering and Technology Research Center for Preservation of Agricultural Products (Tianjin), Institute of Agricultural Products Preservation and Processing Technology, Tianjin Academy of Agricultural Sciences, Tianjin 300384, China
[3] Liaoning Inspection, Examination and Certification Centre, Shenyang 110142, China; hmilyxym333@163.com
[4] Shenyang Institute for Food and Drug Control, Shenyang 110142, China; syspslpy@163.com
* Correspondence: lijkuo@sina.com (J.L.); lidonghua2004168@163.com (D.L.)

**Abstract:** In this study, we aimed to optimize the process of extracting essential oils from *Ligusticum jeholense* Nakai et Kitag. vegetables (LJ-Vs) by means of hydrodistillation (HD), analyze the essential oils' chemical composition, and evaluate their antioxidant and antibacterial activities so as to provide a certain research basis for their development and utilization. A single-factor test and the response surface methodology (RSM) were used to optimize the essential oil extraction process. The chemical constituents of the LJ-V essential oils were analyzed via GC-MS, and the antibacterial and antioxidant activities of the oils were studied. The optimal extraction process conditions were as follows: a solid–liquid ratio of 1:16.3 g/mL, a soaking time of 120 min, and crushing using a mesh size of 40. The validation result for the optimized process was 0.872%. A total of 32 chemical components were detected in LJ-V essential oils, among which the main components were neocnidilide, myristicin, elemicin, and germacrene. LJ-V essential oils with a 20% volume concentration had obvious inhibitory effects on four tested bacteria. The effect on *Staphylococcus aureus* was stronger than that on others. When the dilution concentration exceeded 100 times, the antibacterial effect was not ideal. The sensitivity of the tested bacteria to the essential oils followed the order *Staphylococcus aureus* > *Salmonella* > *Pseudomonas fluorescens* > *Escherichia coli*. Further, LJ-V essential oil had an ideal capacity to scavenge free radicals when compared to Vc control groups. Under the optimized conditions, the essential oil extraction rate was higher, and the process was stable and feasible. This study could provide theoretical and technical support for the cultivation, comprehensive development, and processing of *Ligusticum jeholense* Nakai et Kitag. resources.

**Keywords:** *Ligusticum jeholense* Nakai et Kitag.; essential oil; response surface methodology; antioxidant; antimicrobial activity; GC-MS

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## 1. Introduction

*Ligusticum jeholense* Nakai et Kitag. (*Ligusticum jeholense* or LJ) is a unique and perennial medicinal herb of the Umbelliferaceae family and is found in China. According to the 2020 edition of the *Chinese Pharmacopoeia*, *Ligusticum jeholense* roots are traditional Chinese herbs and have the function of dispersing wind–cold–dampness, along with anti-inflammatory,

bacteriostatic, vasodilating, liver-protecting, and whitening effects [1]. The main chemical components of *Ligusticum jeholense* roots are ferulic acid, volatile oil, ligusticum lactone, and other bioactive substances. They are often used to treat common wind–cold, cold–damp mulligrubs, and arthrodynia [2,3]. *Ligusticum jeholense* is widely distributed in the three eastern provinces of China. Fuxiangyeqin No. 1 is an excellent new variety of *Ligusticum* that was selected and bred by the Fushun Academy of Agricultural Sciences. This variety has been used as a raw medicine and food homology plant for many years; its stems and leaves can be processed as vegetables, and its roots can be used as medicinal materials [4,5]. It is widely planted in Liaoning Province.

Essential oils ($EO_S$) are a group of interesting natural products that have been applied as antimicrobial and antioxidant agents for food security, replacing chemical and synthetic drugs as natural alternatives [6,7]. The volatile oils used in traditional Chinese medicine, which are obtained from traditional Chinese medicine or medicine and food homology plants, have been reported to have anti-inflammatory, antioxidant, oxygen free-radical scavenging, and other pharmacological effects, and they have demonstrated good anti-inflammatory effects in clinics and in experiments [8].

Most of the relevant studies on *Ligusticum jeholense* have focused on the chemical composition of the roots and stems, the potential for medicinal materials, and planting and cultivation [2,5,9]. There have been few studies on essential oils from *Ligusticum jeholense* leaves and stems. The volatile oils of the four kinds of ligusticum *(Ligusticum jeholense*, *Ligusticum sinensis*, *Conioselinum tataricum*, and Mongolian Medicine *Ligustici rhizoma*) (obtained from the roots of these materials) all contain pinene, terpinene, phthalide, neocnidilide, and myristicin [4,10]. Some functional ingredients have also been reported in the ethanol extracts of LJ roots, such as ligustilide, ferulic acid, *Ligustrum* triterpene, and daucosterol [4,5,11]. *Ligusticum jeholense* vegetables (LJ-Vs) are rich leaf and stem resources that can be harvested from different growth periods so it is of great significance to conduct comprehensive research on them.

Several techniques are used for the extraction of essential oils, such as the classic distillation of water vapor, percolation, fluid extraction, and the supercritical fluid extraction method. According to recent findings, the qualitative and quantitative compositions of essential oils are affected by different extraction methods [12,13]. Hydrodistillation (HD) is presented as a traditional plant essential oil extraction method with the advantages of simple equipment requirements, safe operation, high purity of the essential oil, no solvent residues, no environmental pollution, and a low cost, so it has been widely used in essential oil studies [13,14]. It is important to indicate the extraction techniques, the extraction conditions, and the plant part used to explain the variations in EO yields obtained in different studies [15].

The response surface methodology (RSM) is an effective and powerful statistical method that has been used to optimize the essential oil extraction process [15]. Although fewer experimental runs are conducted when using the RSM than when using a full factorial design, statistically acceptable results can still be provided. Therefore, the RSM is a set of statistical and numerical methods that can be efficiently utilized to develop, enhance, and optimize processes [16,17].

In this study, the RSM was used to optimize the extraction conditions and improve the extraction yield of *Ligusticum jeholense.* Gas chromatography–mass spectrometry (GC-MS) was used to analyze the chemical components of essential oils obtained from the stems and leaves of LJ-Vs. The antibacterial effect and antioxidant activity of the essential oils were preliminarily studied in order to provide a scientific basis for the deep processing and application of *Ligusticum jeholense* vegetables.

## 2. Materials and Methods

### 2.1. Plant Materials

*Ligusticum jeholense* vegetables were collected in June 2024 at the Herbal Medicine planting base of Yingyimen in Qingyuan County, Fushun City, Liaoning Province. The plant material was identified by senior agrologist Pan Yiyuan, and voucher specimens were stored in the herbarium of the College of Environmental and Safety Engineering, Shenyang University of Chemical Technology. The fresh plants were dried in an oven at 40 °C and reserved for later use.

### 2.2. Extraction Method

#### 2.2.1. Extraction of Essential Oil

Dried *Ligusticum jeholense* stems (LJ-Ss), *Ligusticum jeholense* leaves (LJ-Ls), and whole *Ligusticum jeholense* vegetables (LJ-Vs) were individually crushed, and the corresponding essential oils were extracted by means of steam distillation using Clevenger-type apparatuses. The specific steps were as follows: A quantity of 10 g of the powdered material was macerated in 140 mL of distilled water at ambient temperature (25 °C) for 2 h with occasional agitation. The volatile oils were extracted until no more oil was obtained in the apparatus, and they were dried with anhydrous $Na_2SO_4$. At the end of this period, the essential oil was collected in a reagent bottle with a plug. The essential oils were obtained in triplicate for each oil sample, and the average yield was calculated. The formula that was used to calculate the EO yield is as follows (Equation (1)):

$$\text{EO yield\%} = (m_1/m_0) \times 100 \quad (1)$$

where $m_1$ is the weight of EO (g) and $m_0$ is the initial weight of the powder (g).

#### 2.2.2. Single-Factor Experimental Design

On the basis of previous experiments and with reference to the relevant literature [14–16], this experiment determined that the grinding particle size, soaking time, and solid–liquid ratio were the main factors affecting the extraction of essential oil from Ligusticum Jeholense wild vegetables by means of steam distillation. The essential oil yield was taken as the investigation index, and a crushing particle size of 40 mesh, solid–liquid ratio (weight of *Ligusticum jeholense* plant powder to volume of distilled water) of 1:16 g/mL, and soaking time of 120 min were taken as the fixed baseline values. Then, the individual effects of various crushing sizes (20, 40, 60, 80, 100 mesh), solid–liquid ratios (1:12, 1:14, 1:16, 1:18, 1:20 g/mL), and soaking times (60, 90, 120, 150, 180 min) on the yield of *Ligusticum jeholense* vegetable essential oil were determined. The experiments were repeated 3 times for each group.

#### 2.2.3. Response Surface Test Design

On the basis of the single-factor tests, with the pulverized particle size, solid–liquid ratio, and soaking time as independent variables and the essential oil yield as the response value, a Box–Behnken response surface with three factors and three levels was selected for analysis, as shown in Table 1.

**Table 1.** Response surface design factors and levels.

| Factor Level | (A) Grinding Size (mesh) | (B) Solid–Liquid Ratio (g/mL) | (C) Soaking Time (min) |
|---|---|---|---|
| −1 | 20 | 1:14 | 90 |
| 0 | 40 | 1:16 | 120 |
| 1 | 60 | 1:18 | 150 |

### 2.3. GC-MS Analysis

The pretreatment of essential oil was as follows: Samples of 25 mg of the three kinds of LJ-V essential oil were placed in 50 mL volume bottles, and mother oils were prepared with ethyl acetate. After 1 mL of mother oil was diluted 10 times, 2 mL of diluent was placed in a sample bottle for machine determination.

The gas chromatographic conditions were as follows: An HP-5MS elastic quartz capillary column (column length: 30 m × 0.25 mm × 0.25 μm) was used, and the temperature program was as follows: The initial temperature was 40 °C and was raised at 5 °C/min to 70 °C; then, the temperature was raised at 15 °C/min to 250 °C and maintained for 2 min. The inlet temperature was 250 °C, the sample size was 1 μL, and the sample was injected by means of a shunt. The shunt ratio was 30:1, the solvent delay was 10 min, the carrier gas was helium, and the carrier gas flow rate was 1.0 mL/min.

The mass spectrum conditions were as follows [18]: electron bombardment (EI) ion source; electron energy, 70 eV; GC and MS interface temperature, 280 °C; ion source temperature, 230 °C; electron detector detection voltage, 1282 V; scanning mass range ($m/z$), 50–350 amu; mass spectrometry standard library, NIST14 mass spectrometry library.

### 2.4. Antibacterial Activity

The in vitro antibacterial activity of the LJ-V essential oils was assessed against *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas fluorescens* (ATCC 13525), representing Gram-positive bacteria, and *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028), representing Gram-negative bacteria.

The preparation of plates containing bacteria was as follows [19,20]: The antibacterial effect of the LJ-V essential oils was measured by means of disk diffusion. An activated test strain was selected from inclined planes, inoculated into a 25 mL nutrient broth liquid medium, and cultured in a shaking table at 37 °C for 18–24 h, such that the strain entered the logarithmic growth stage. After culture, the tested strains were centrifuged at 4500 r/min at 10 °C for 15 min; the supernatant was discarded, and the remainder was diluted with sterile normal saline into a bacterial suspension with a concentration of $10^6$-$10^8$ cfu/mL. A 300 μL sample of the bacterial suspension was pipetted onto a plate, which was uniformly coated to make a bacterial plate.

Determination of inhibition zones: LJ-V essential oils were diluted with ethyl acetate to volume concentrations of 20%, 10%, 2%, 1%, and 0.1% ($v/v$%). A 10 mm filter paper was placed on the bacterial plate, and 20 μL of diluted essential oil was dropped onto the filter paper. Ethyl acetate was used as the control, and the plates were incubated at a constant temperature for 18–20 h. The diameter of the inhibition zone was observed and measured [20,21].

### 2.5. Antiradical Activity

Five concentrations of LJ-V concentrate were prepared—0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL—and their antioxidant activities were determined.

#### 2.5.1. DPPH Radical Scavenging Activity

The method used to determine 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was slightly modified based on references [14,22]. Essential oils of different mass concentrations were mixed with 3 mL of 0.2 mmol /L DPPH solution. $A_1$ was taken as the absorbance measured at a 517 nm wavelength after a reaction time of 30 min, $A_0$ was taken as the absorbance determined with anhydrous ethanol instead of essential oil, and $A_2$ was the absorbance determined with anhydrous ethanol instead of DPPH solution.

All experiments were performed in triplicate, and the DPPH radical scavenging rate was calculated using $V_C$ as a positive control. The formula was as follows (Equation (2)):

$$\text{DPPH radical scavenging \%} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (2)$$

#### 2.5.2. ABTS Radical Scavenging Activity

*2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid* (ABTS) mother liquor was prepared according to a method described in the literature [23]. Volumes of 3 mL each of essential oils with different mass concentrations and ABTS working liquid were thoroughly mixed. $A_1$ was taken as the absorbance determined after the reaction was conducted for 30 min in the dark, $A_0$ was taken as the absorbance determined with anhydrous ethanol instead of essential oil, and $A_2$ was taken as the absorbance determined with anhydrous ethanol instead of ABTS solution. The essential oil experiments were performed in triplicate for each concentration. The average value was taken, using $V_C$ as a positive control, and the ABTS radical scavenging rate was calculated using the formula in Equation (2).

#### 2.5.3. OH Radical Scavenging Activity

Following a method from [24], with modifications, 3 mL volumes of essential oil with different mass concentrations were taken, and 0.5 mL volumes of each solution of ferrous sulfate, salicylic acid, and hydrogen peroxide were added to the essential oil in a water bath at 37 °C for 30 min. $A_1$ was taken as the absorbance value measured at a 510 nm wavelength, $A_2$ was taken as the absorbance determined with anhydrous ethanol instead of salicylic acid, and $A_0$ was taken as the absorbance determined with anhydrous ethanol instead of the essential oil test solution. All experiments were performed in triplicate. $V_C$ was used as the positive control to calculate the clearance rate of OH radicals. The formula was the same as that in Equation (2).

### *2.6. Data Processing*

The tests in each group were repeated three times, and the average results were taken. Origin 2019b software was used to draw the single-factor test diagram, Design-Expert 8.0 software was used to draw the response surface diagram, and SPSS 27.0 software was used for probability analyses and calculations.

## 3. Results

### *3.1. Yield and Sensory Properties of Ligusticum jeholense Essential Oil*

According to the analysis of the results in Table 2, there was no significant difference in the yields of Lj-L and LJ-V essential oils, obtained from the leaves and whole vegetables, respectively ($p < 0.05$), while the yield of LJ-S essential oil was lower. Stems are the guiding tissues of plants, so components are less abundant in stems than in other parts. In a similar study, Ljubomir Š. et al. analyzed the essential oil contents of wild sea fennel and found that the leaves contained less EO than did immature inflorescences. The minimum EO content was found in the stems [25]. We also observed that the LJ-L essential oil was a slightly darker color than the other two oils, and there was no significant difference in odor among the three essential oils. Their sensory properties indicate that they would be pleasant, widely used essential oils. Essential oils (EOs) have numerous commercial applications due to their diverse biological properties and appealing fragrances. They can be used to produce a wide variety of pharmaceutical products, such as gels, creams, ointments, nano-emulsions, and patches [26,27].

**Table 2.** The yield and sensory properties of *Ligusticum jeholense* essential oils from different plant parts.

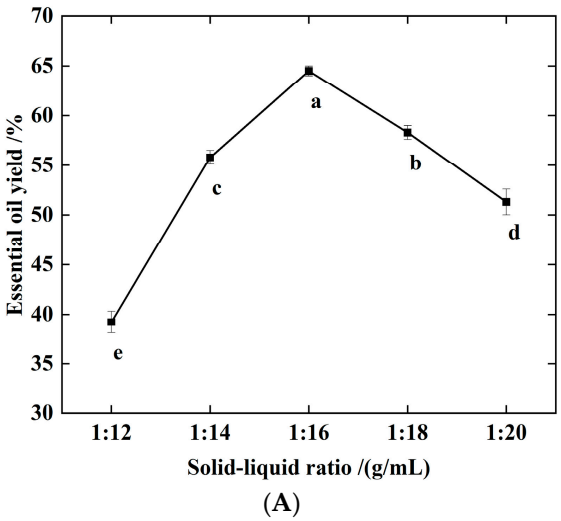
| Index | LJ-L Essential Oil | LJ-S Essential Oil | LJ-V Essential Oil |
|---|---|---|---|
| yield (%) | 0.557 ± 0.005% a | 0.417 ± 0.017% b | 0.529 ± 0.026% a |
| appearance | Transparent, light yellow, oleous | Transparent, light yellow, oleous | Transparent, light yellow, oleous |
| odor | Vegetable and Chinese medicine mixed smell | Vegetable and Chinese medicine mixed smell | Vegetable and Chinese medicine mixed smell |

Different lowercase letters after yield data indicate a significant difference ($p < 0.05$); LJ-L: *Ligusticum jeholense* leaf; LJ-S: *Ligusticum jeholense* stem; LJ-V: *Ligusticum jeholense* vegetable.

Considering the essential oil yields, the convenience of pretreatment, and practical operability, the experiments in this study determined that drying whole plants is the appropriate pretreatment method; thus, the LJ-V essential oil extraction process was optimized to improve the yield.

### *3.2. Analysis of the Influence of Single Factors on the Extraction of Essential Oil from Ligusticum jeholense Vegetables*

The influence of the solid–liquid ratio on the yield of LJ-V essential oil is shown in Figure 1A. When the volume of distilled water is too large or too small, the active ingredient cannot be fully released. Therefore, the appropriate solid–liquid ratio was determined to be 1:16 (g/mL). The influence of the soaking time on the yield of LJ-V essential oil is shown in Figure 1B. With the extension of the soaking time, the amount of extracted volatile oil first increased and then decreased. When the soaking time exceeded 2 h, due to the volatilization and loss of essential oil, the amount of extracted volatile oil decreased, so 120 min was determined as the most appropriate soaking time. When the fineness exceeded 60 mesh, clumping blockages occurred during the reaction process, and the yield of essential oil gradually decreased. Therefore, 40 mesh was determined as the most suitable grinding fineness, as shown in Figure 1C.




(**A**)

**Figure 1.** *Cont.*

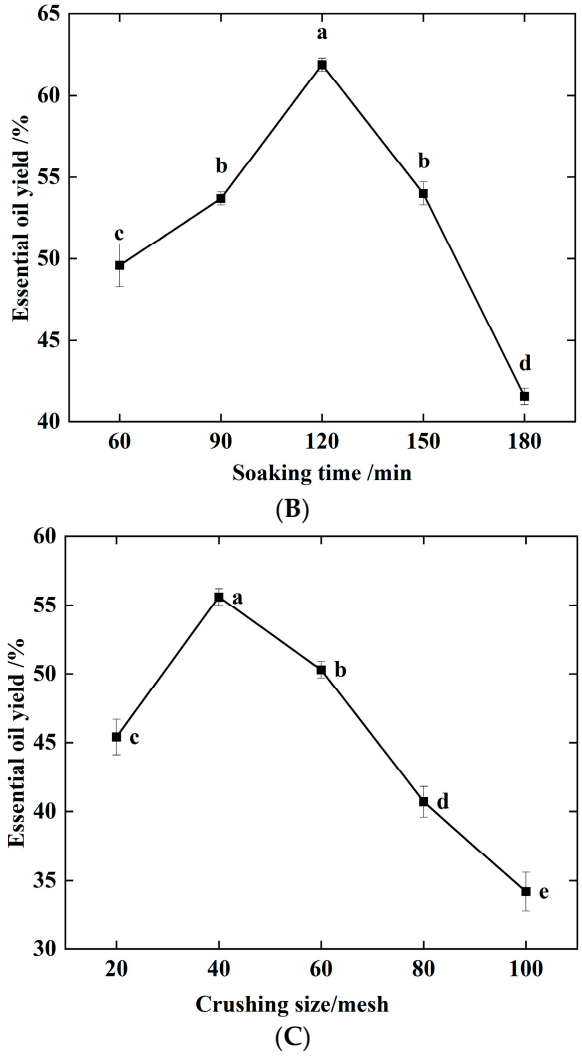



**Figure 1.** The effects of the solid–liquid ratio (**A**), soaking time (**B**), and particle size (**C**) on the yield of essential oil from *Ligusticum jeholense* vegetables. Different lowercase letters indicated a significant difference ($p < 0.05$), while the same letter indicated no significant difference ($p > 0.05$).

### *3.3. Response Surface Test Design and Results*

#### 3.3.1. Design Scheme and Results of Box–Behnken Experiments

A response surface analysis of 17 test sites with three factors and three levels was conducted with the extraction rate of essential oil from *Ligusticum jeholensetian* vegetables as the response value. The design combination and results are shown in Table 3.

**Table 3.** Experimental design and results of the Box -Behnken test.

| Numbering | *A* | *B* | *C* | Yield/% | Numbering | *A* | *B* | *C* | Yield/% |
|---|---|---|---|---|---|---|---|---|---|
| 1 | −1 | −1 | 0 | 0.64 | 10 | 0 | 1 | −1 | 0.67 |
| 2 | 1 | −1 | 0 | 0.73 | 11 | 0 | −1 | 1 | 0.63 |
| 3 | −1 | 1 | 0 | 0.75 | 12 | 0 | 1 | 1 | 0.76 |
| 4 | 1 | 1 | 0 | 0.72 | 13 | 0 | 0 | 0 | 0.86 |
| 5 | −1 | 0 | −1 | 0.69 | 14 | 0 | 0 | 0 | 0.87 |
| 6 | 1 | 0 | −1 | 0.70 | 15 | 0 | 0 | 0 | 0.85 |
| 7 | −1 | 0 | 1 | 0.72 | 16 | 0 | 0 | 0 | 0.86 |
| 8 | 1 | 0 | 1 | 0.79 | 17 | 0 | 0 | 0 | 0.87 |
| 9 | 0 | −1 | −1 | 0.69 | | | | | |

*A:* solid–liquid ratio; *B*: soaking time; *C*: particle size.

3.3.2. Establishment of Regression Model

Following the Box–Behnken response surface analysis, Design expert 10.0 software was used to regression-fit the data in Table 3, and the following quadratic polynomial regression equation was obtained:

$$Y = 0.8620 + 0.0175A + 0.0263B + 0.0188C - 0.03AB + 0.150AC + 0.0375BC - 0.0573A2 - 0.0948B2 - 0.0798C2 \quad (3)$$

The variance analysis results for this regression model are shown in Table 4.

**Table 4.** Analysis of variance of the regression model.

| Source of Variance | Sum of Squares | Degree of Freedom | Mean Deviation | F Value | *p* | Significance |
|---|---|---|---|---|---|---|
| Model | 0.1169 | 9 | 0.0130 | 61.99 | <0.0001 | ** |
| A | 0.0002 | 1 | 0.0024 | 12.66 | 0.0092 | ** |
| B | 0.0055 | 1 | 0.0055 | 28.48 | 0.0011 | ** |
| C | 0.0028 | 1 | 0.0028 | 14.53 | 0.0066 | ** |
| AB | 0.0036 | 1 | 0.0036 | 18.60 | 0.0035 | ** |
| AC | 0.0009 | 1 | 0.0009 | 4.65 | 0.0680 | |
| BC | 0.0056 | 1 | 0.0056 | 29.06 | 0.0010 | ** |
| $A^2$ | 0.0158 | 1 | 0.0158 | 71.29 | <0.0001 | ** |
| $B^2$ | 0.0411 | 1 | 0.0411 | 195.28 | <0.0001 | ** |
| $C^2$ | 0.0295 | 1 | 0.0295 | 138.34 | <0.0001 | ** |
| Residual error | 0.0017 | 7 | 0.0002 | | | |
| Missing fit | 0.0011 | 3 | 0.0004 | 5.12 | 0.0743 | |
| Pure error | 0.0006 | 4 | 0.0002 | | | |
| Total error | 0.1186 | 16 | | | | |
| | $R^2 = 0.9876$ | $R_{Adj}^2 = 0.9717$ | $R_{pre}^2 = 0.8387$ | | | |

** very significant difference ($p < 0.01$), * significant difference ($p < 0.05$), A: solid–liquid ratio; B: soaking time; C: particle size.

As can be seen from Table 4, the F value of the model is 61.99, $p < 0.0001$, indicating that the model has extremely high statistical significance. The $p$ value of the missing fit item is 0.0743 > 0.05; therefore, the missing fit item is not significant, indicating that unknown factors interfered little with the test results. The $R^2$ correlation coefficient of the model is 0.9876, indicating that the model has high significance and can interpret 97.17% of the response value change ($R_{Adj}^2 = 0.9717$). The $R_{pre}^2$ correlation coefficient is 0.8387, and the difference between this and $R_{Adj}^2$ (0.133) is small. The coefficient of variation (CV) of the model is 1.85% < 10%, and the noise ratio is 21.16% > 4%, indicating that the experimental values of the regression model have high accuracy and reliability [28,29]. The primary terms A, B, and C, interaction terms AB and BC, and secondary terms $A^2$, $B^2$, and $C^2$ have significant effects on the yield of essential oil ($p < 0.01$), while the interaction term AC has no significant effect on the yield. According to the F values, the yield of essential oil is affected by the three primary factors in the order of B (soaking time) > C (crushing particle size) > A (solid–liquid ratio).

3.3.3. Contour and Response Surface Analysis

The response surface and contour maps of various factors can reflect the interactions among them. The greater the slope of the response surface curve, or the closer the contour map is to an oval shape, the greater the influence of the factor on the response value [16,29]. As can be seen from Figure 2, the contour plots among all factors were elliptical, the response surface was steep, and the yields of essential oil from the stem and leaves of Liaousticum presented extreme response values in the test range corresponding to the three significant influencing factors. The soaking time (B) has a significant effect on the yield of

essential oil, and its curve is relatively steep, which is consistent with the results in Table 4. In the figure, the 3D maps of AB and BC are steeper, and the contour shapes are obviously elliptical, indicating that the interactions represented by the interaction terms AB and BC have a significant impact on the yield of essential oils. In the figure, the response surface map of AC has a gentle slope and no obvious oval shape, indicating that the interaction represented by AC has no significant impact on the yield of essential oils; this is consistent with the results of the variance analysis of the regression model, presented in Table 4.

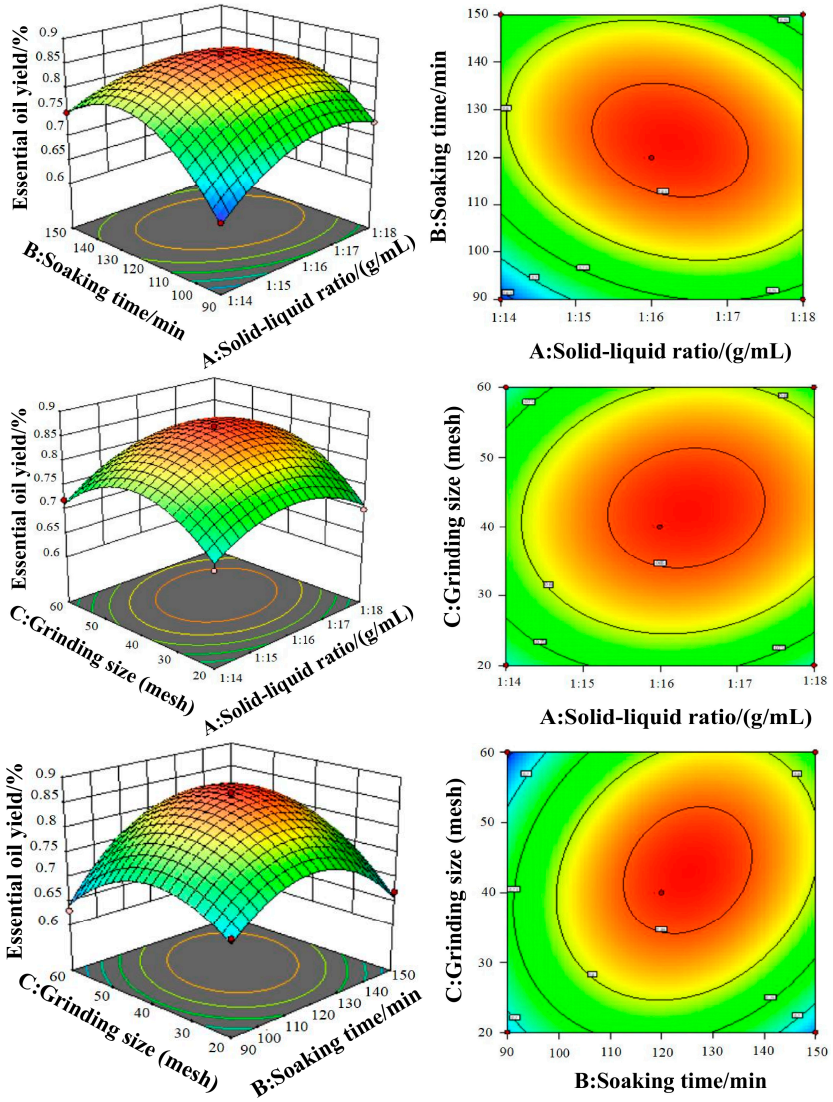



**Figure 2.** Response surface diagrams and contour maps showing the effects of interactions of various factors on the extraction yield of LJ-V essential oil.

### 3.3.4. Validation Test

The regression model predicted that the optimal extraction process involved the following parameters: a solid–liquid ratio of 1:16.271 g/mL, a soaking time of 124.481 min, and a grinding particle size of 43.316 mesh. Under such conditions, the yield predicted by the model was 0.867%. Considering the feasibility of these settings in the actual process, the extraction conditions were revised to a solid–liquid ratio of 1:16.3 g/mL, a soaking time of 125 min, and a grinding particle size of 40 mesh. According to these optimized conditions, three validation experiments were conducted. The average yield of essential oil was 0.872%, with a small relative error (0.57%) compared with the expected value of

0.867%, indicating that the established model was reliable and could provide a reference for the efficient extraction of essential oil from *Ligusticum jeholense* vegetables.

### *3.4. Results of GC-MS Analysis*

A total of 43 components were detected by means of GC-MS. The peaks with matching degrees of more than 90% were identified as candidate compounds. The mass spectrograms of each peak were calculated and searched in a mass spectrogram database [4,5,9,11], and the mass spectrogram of each peak was compared with the literature to identify 32 components. The specific results are shown in Table 5.

**Table 5.** Composition analysis of *Ligusticum jeholense* essential oil by means of GC-MS.

| Serial Number | Component Name | Molecular Formula | Relative Content% | | |
|---|---|---|---|---|---|
| | | | LJ-V Essential Oil | LJ-S Essential Oil | LJ-L Essential Oil |
| 1 | *(-)*-Carvyl dihydroacetate | $C_{12}H_{20}O_2$ | - | - | 0.54 |
| 2 | *Cis-beta*-farnesene | $C_{15}H_{24}$ | - | 0.67 | - |
| 3 | *(-)-alpha*-cubebeene | $C_{15}H_{24}$ | - | - | 0.15 |
| 4 | *(-)-alpha*-pinene | $C_{15}H_{24}$ | 0.41 | 0.48 | 0.70 |
| 5 | 8-isopropenyl-1, 5-dimethyl-cyclodecane, 5-diene | $C_{15}H_{24}$ | 3.04 | 1.79 | 4.16 |
| 6 | *trans*-caryophyllene | $C_{15}H_{24}$ | 4.18 | 1.38 | 5.59 |
| 7 | *(E)*-beta-farnesene | $C_{15}H_{24}$ | 0.68 | 0.32 | 2.01 |
| 8 | *alpha*-caryophyllene | $C_{15}H_{24}$ | 1.32 | 0.23 | 1.49 |
| 9 | *alpha*-itranolene | $C_{15}H_{24}$ | 0.21 | - | 0.38 |
| 10 | germacrene | $C_{15}H_{24}$ | 9.88 | 1.53 | 7.48 |
| 11 | *beta*-celene | $C_{15}H_{24}$ | 1.21 | 2.32 | 1.24 |
| 12 | [4aR-(4aa,7a,8aβ)]-Decahydro-4a-methyl-1-methylene-7-(1-methylvinyl-naphthalene) | $C_{15}H_{24}$ | 1.12 | 1.47 | 1.01 |
| 13 | *alpha*-farnesene | $C_{15}H_{24}$ | - | - | 1.46 |
| 14 | γ-guassene | $C_{15}H_{24}$ | 3.73 | - | 2.14 |
| *15* | *Trans*-isomethyl ether | $C_{11}H_{12}O_3$ | 1.18 | 1.88 | |
| 16 | β-cadinene | $C_{15}H_{24}$ | 2.88 | - | 3.73 |
| 17 | *(-)-Trans*-pinocarvol | $C_{15}H_{24}O$ | 0.20 | 0.24 | - |
| 18 | Myristic aldehyde | $C_9H_8O_4$ | 0.76 | 0.66 | - |
| 19 | Cinetol | $C_{15}H_{24}O$ | 1.21 | 5.78 | 1.25 |
| 20 | caryophyllin | $C_{15}H_{24}O$ | 4.19 | 3.16 | 3.69 |
| 21 | Myristin | $C_{11}H_{12}O_3$ | 12.46 | 13.42 | 18.68 |
| 22 | elemicin | $C_{12}H_{16}O_3$ | 8.56 | 9.40 | 3.77 |
| 23 | *T*-cadinol | $C_{15}H_{26}O$ | - | - | 2.71 |
| 24 | *A*-Pecoranol | $C_{15}H_{26}O$ | 2.16 | 2.02 | 2.4 |
| 25 | 2,6-di-tert-butyl-1,4-resorcinol | $C_{14}H_{22}O_2$ | 0.74 | 1.03 | 0.32 |
| 26 | 4(15),5,10(14)-macrorhizotriene-1-ol | $C_{15}H_{24}O$ | 0.92 | 1.64 | - |
| 27 | β-Asaryl ether | $C_{12}H_{16}O_3$ | 2.14 | 0.65 | 1.43 |
| 28 | Neocnidilide | $C_{12}H_{18}O_2$ | 32.17 | 43.56 | 28.59 |
| 29 | Celery camphor | $C_{12}H_{14}O_4$ | 1.51 | 1.44 | 1.56 |
| 30 | 8,9-epoxidone-B | $C_{15}H_{24}O_2$ | 0.27 | - | 0.36 |
| 31 | boral | $C_{14}H_{22}O$ | 0.41 | 0.43 | 0.19 |
| 32 | 2-methyl-4-[2′,6′,6′-trimethyl-1′-cyclohexene-1′-yl]-3-butene-1-ol | $C_{14}H_{24}O$ | 0.29 | 0.48 | 0.18 |

As shown in Table 5, GC-MS was used to analyze EOs from *Ligusticum jeholense*. For LJ-V, 27 components were identified, accounting for 97.83% of the total essential oil; for LJ-S, 24 components were identified, accounting for 95.98% of the total essential oil; and for LJ-L, 27 components were identified, accounting for 97.21% of the total essential oil. Nineteen components were shared by the three essential oils. Among them, neocnidilide (32.17% in LJ-V, 43.56% in LJ-S, 28.59% in LJ-L), myristin (12.46% in LJ-V, 13.42% in LJ-S, 18.68% in LJ-L), elemicin (8.56% in LJ-V, 9.4% in LJ-S, 3.7 in LJ-L, 7%), and germacrene (9.88% in LJ-V, 1.53% in LJ-S, 7.48% in LJ-L) were the representative components (with average contents above 5%). Li S. et al. showed that phthalides (i.e., neocnidilide) and phenylpropanoids (i.e., myristicin) can be thought of as the most critical components in the volatile oils of *Ligusticum jeholense Nakai et Kitag.* (LJ) and *Ligusticum sinese oliv.* (LS) [4],

which is consistent with our experimental results. The classification and analysis results for each component type are shown in Figure 3.

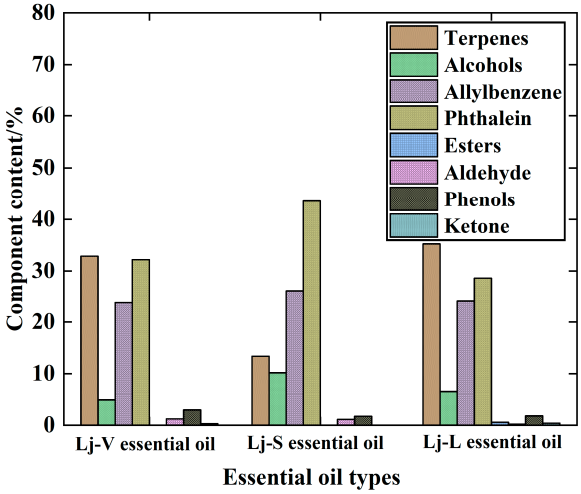



**Figure 3.** Component type and quantity analysis of three *Ligusticum jeholense* essential oils.

As can be seen from Figure 3, the main components of LJ essential oils obtained from three different plant parts were benzene phthalides, allylbenzenes, and terpene compounds. The analysis of allylbenzene compounds (myristicin, olive, and trans-isomethyl) showed that the differences between the three essential oils were not significant, and the terpene compound content of LJ-L essential oil was the highest (35.23%). LJ-S essential oil had the highest content of phthalides (43.56%), while LJ-V essential oil contained terpenes (32.85%), phthalides (23.71%), and neocnidilide (32.17%). Thus, the EO contents of LJ-V essential oil were satisfactory, which may be due to its extraction from whole vegetables. The proportions of stems and leaves were suitable (the mass fractions of stems and leaves were 23.8 $\pm$ 5.5% and 76.2 $\pm$ 4.5%, respectively), and the types and contents of active components were abundant in LJ-V essential oil.

The results of GC-MS showed that the main terpenes in the essential oil were sesquiterpenes, with very few monoterpenes. Mansour Miran et al. [30], Wang et al. [11], and Li S. et al. [4] detected the chemical components of volatile oils from several ligusticum plants, and they pointed out that $\beta$-Phellandrene was one of the main components. No monoterpenoids such as β-hydroconene were detected in this experiment, which may be due to the use of different extraction sites and extraction methods for ligusticum raw materials. Mansour Miran et al. reported that pentylcyclohexa-1,3-diene, (Z)-ligustilide, and neocnidilide are major compounds in aerial parts at the flowering and fruiting stages, while these compounds are minor in roots at the flowering or fruiting stage, and that the $\beta$-Phellandrene content is higher in roots than in aerial parts [30]. This study focused on wild vegetables.

The results of GC-MS showed that there were other bioactive components in the *Ligusticum jeholense* vegetables, such as caryophyllene, germacrene, and cadinene [31].

### *3.5. Analysis of Bacteriostatic Effect of Ligusticum jeholensetian Wild Vegetable Essential Oil*

Based on the GC-MS results above, the foodborne pathogenic bacteria Staphylococcus aureus and Salmonella and the food-rot bacteria Escherichia coli and Pseudomonas fluorescens were selected as the object bacteria in this experiment to study the antibacterial activity of LJ-V essential oil. The specific results are shown in Table 6.

**Table 6.** Antibacterial activity of LJ-V essential oil obtained from whole wild vegetables.

| Concentration of Essential Oil by Volume (*v*/*v*%) | Inhibition Zone Diameter d/mm | | | |
|---|---|---|---|---|
| | ***Staphylococcus aureus*** | ***Pseudomonas fluorescens*** | ***E. coli*** | ***Salmonella*** |
| 20% | 16.96 ± 0.59 [a] | 12.06 ± 0.28 [a] | 11.98 ± 0.43 [a] | 13.62 ± 0.14 [a] |
| 10% | 13.37 ± 0.49 [b] | 11.36 ± 0.35 [b] | 11.19 ± 0.13 [b] | 12.85 ± 0.45 [ab] |
| 2% | 11.42 ± 0.15 [c] | 11.04 ± 0.25 [b] | 10.94 ± 0.11 [b] | 11.94 ± 0.32 [b] |
| 1% | 11.22 ± 0.08 [c] | 10.16 ± 0.09 [c] | - | - |
| 0.1% | 11.06 ± 0.04 [c] | 10.16 ± 0.05 [c] | - | - |
| Ethyl acetate | 10.12 ± 0.06 [d] | 10.14 ± 0.05 [c] | - | - |

Superscript [a], [b], [c], [d] indicate significant differences in the diameter of the antibacterial zone under different concentrations ($p < 0.05$).

As can be seen from Table 6, the ethyl acetate solvent had almost no antibacterial activity against *Salmonella* and *Escherichia coli*, while it had weak antibacterial activity against *Pseudomonas fluorescein* and *Staphylococcus aureus*. Therefore, it was feasible to select ethyl acetate as the dissolving reagent for LJ-V essential oil. LJ-V essential oil with a 20% concentration by volume showed an obvious inhibitory effect on all four of the tested strains. The inhibitory effect on *Staphylococcus aureus* was the most prominent (d = 16.96 ± 0.59 mm), and the inhibitory effect on *Escherichia coli* was the weakest (d = 11.98 ± 0.43 mm), which may be because *Escherichia coli* is a typical Gram-negative bacterium. It has a strong defense ability against plant essential oil, as reported in antibacterial tests of levisticum officinale essential oil [30], rosemary essential oil [32], and salvia essential oil [33].

The antibacterial ability of the essential oil decreased with an increase in the dilution ratio, and the antibacterial effect at a volume of <1% was not ideal. At the same LJ-V essential oil concentration, the sensitivity of the different strains to the oil was in the order *Staphylococcus aureus* > *Salmonella* > *Pseudomonas fluorescens* > *Escherichia coli*. The difference between the essential oil and ethyl acetate control group was analyzed, which fully proved the antibacterial effect of LJ-V essential oil.

### *3.6. Analysis of Antioxidant Activity of Essential Oil of Ligusticum jeholensetian Vegetables*

LJ-V essential oil had scavenging effects on all three kinds of free radicals tested in this study, as shown in Figure 4. In the range of 0.2–1.0 mg/mL, there was a dose–effect relationship between the clearance rate and the concentration of essential oil, and the clearance rates of all three kinds of free radicals increased with an increase in the sample concentration. When the concentration was 1 mg/mL, the scavenging rates of the essential oil on DPPH, ABTS, and OH radicals were 73.38%, 69.73%, and 71.35%, respectively, while the scavenging rates of $V_C$ on the same free radicals were 87.40%, 82.41%, and 88.66%. A probability analysis was conducted using SPSS 27.0 software. The $IC_{50}$ values of LJ-V essential oil were 0.566 mg/mL for DPPH, 0.683 mg/mL for ABTS, and 0.599 mg/mL for OH. However, its reducing ability presented a certain gap when compared with that of $V_C$.

A wide variation in the antioxidant activity of *Ligusticum jeholens* was observed in the literature. The DPPH inhibition rates of *L. jeholense* essential oils were 407.38 μg/mL for *L. sinense* and 177.04 μg/mL for *L. jeholens*, respectively, when assayed by Wang et al. [11]. *Ligusticum chuanxiong* essential oil presented strong antioxidant activity, with an $IC_{50}$ value for scavenging DPPH free radicals of 1.014 ± 0.014 mg/mL [34]. The authors pointed out that ligustilide and senkyunolide A were screened as having strong DPPH scavenging properties. The essential oil extracted in this study could be developed and used as a natural antioxidant and synergist in medicine, food, and other fields.

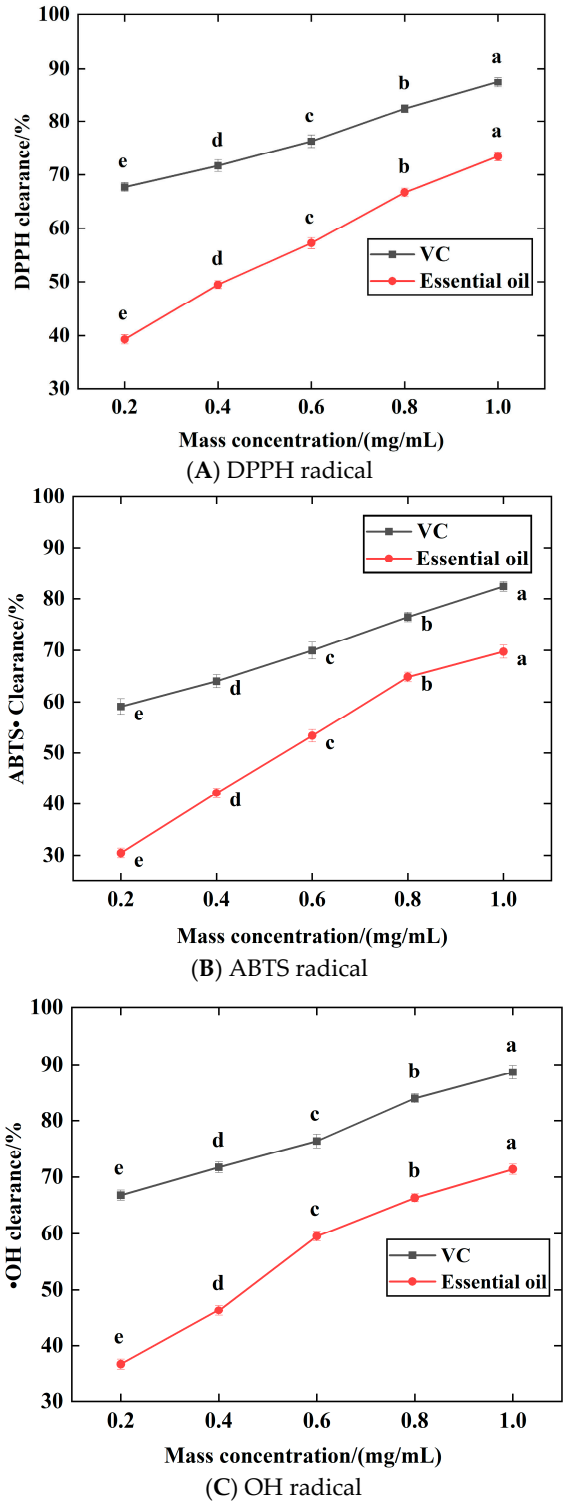



**Figure 4.** Scavenging rates of LJ-V essential oil. Different lowercase letters indicated a significant difference ($p < 0.05$), while the same letter indicated no significant difference ($p > 0.05$).

## 4. Conclusions

In this study, the water steam distillation method was used to extract LJ-V essential oil. Based on single-factor tests, a response surface optimization was carried out to obtain optimal extraction process parameters for LJ-V essential oil: a crushing particle size of 40 mesh, a solid–liquid ratio of 1:16.3 g/mL, and a soaking time of 125 min. Under these conditions, the essential oil yield in the verification test was 0.872%, which was 1.57 times higher than that in the pre-optimization test (0.529 ± 0.026)%. The results indicated that

the extraction conditions obtained by means of response surface optimization were reliable and could provide a technical reference for the efficient extraction of essential oils from LJ vegetables. Thirty-two chemical components of LJ essential oils were identified by means of GC-MS analysis. The main components of the three essential oils were neocnidilide, myristicin, elemicin, and germacrene. The contents of terpenes were the highest in the leaf essential oil, while the stem essential oil had the highest content of phthalides. Antibacterial tests showed that LJ-V essential oil had good antibacterial activity against the four tested strains. The antibacterial activity of the essential oil increased with an increase in the volume percentage, and LJ-V essential oil at 20% volume had the most obvious inhibitory effect on *Staphylococcus aureus*. When the dilution concentration of the essential oil was greater than 100 times, the antibacterial effect was not ideal. The sensitivity of the tested strains to the essential oil was in the order *Staphylococcus aureus* > *Salmonella* > *Pseudomonas fluorescens* > *Escherichia coli*. The antioxidant results showed that LJ-V essential oil has a certain radical scavenging ability, and its antioxidant ability increased with an increase in the concentration of essential oil.

At present, research on plant essential oils from *Ligusticum jeholense* vegetables is still in its primary stage, and the physicochemical properties and physiological activities of these oils need to be further developed. Some research studies have reported that the contents of components in plants vary at different growth stages [25,35]. In the next step of this study, we will systematically collect stems and leaves of *Ligusticum jeholense* at different growth stages for component and activity analyses to explore these patterns. Based on the RSM extraction optimization process, our research team will attempt to apply some auxiliary technologies to improve the extraction rate, such as microwave, ultrasonic, and enzymatic technologies [13,24]. The research on *Ligusticum jeholense* essential oil's antimicrobial activity will be expanded to study its effects on common spoilage microorganisms such as fungi and yeasts [36].

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