Article

Discovery of Quality by Design-Based Extraction Technology and the Quality Standard of Jinhua Finger Citron Essential Oil

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ABSTRACT: Jinhua finger citron is one of the traditional specialties of Jinhua with a long history of cultivation. The current study highlighted the advantages of using the quality by design approach to optimize the ultrasonic-assisted distillation extraction of Jinhua finger citron essential oil. The yield of Jinhua finger citron essential oil was regarded as a potential critical quality attribute. Potential critical process parameters were screened by the definitive screening design. Finally, the design space of the essential oil extraction process was constructed. The optimal operating space included an auxiliary NaCl concentration range of 9−12.00%, a soak temperature range of 30−50 °C, a distillation time range of 3.5−4.00 h, an ultrasonic power range of 200−300 W, a solid−liquid ratio range of 1:3−1:3.5, and a soak time range of 40−80 min. On this basis, the relative density, refractive index, and pH values of different batches of Jinhua finger citron essential oil were checked. The involved batches were analyzed by gas chromatography (GC), and gas chromatographic fingerprints were established by identifying major compounds, including D-limonene, γ-terpinene, and 5,7-dimethoxycoumarin. Based on the "quality by design" strategy, the extraction process of Jinhua finger citron essential oil established in this study was robust, reliable, and flexible.

1. INTRODUCTION

Finger citron (*Citrus medica* L. var. *sarcodactylis* Swingle) is one of the variants of *C. medica* L. As the fruit grows, the branches separate to produce finger-like fructification, hence named "finger citron."[1] Due to distinct four seasons and a warm and humid climate, as well as abundant sunshine in the Jinhua area, the cultivated finger citron is known as a "Celestial Fruit, Exotic Plant."[2] Jinhua finger citron (JFC), praised as the "Golden Finger Citron," enjoys a long history of cultivation. The main functions include regulating qi-flowing for eliminating phlegm, relieving cough, reducing flatulence, soothing the liver, and strengthening the spleen.[3,4]

The essential oil extracted from the fruit of finger citron is usually a transparent, yellowish, and fragrant product. Studies have shown that essential oils have good performances on antibacterial utilization, promoting insulin secretion, inhibiting cholesterol synthesis, and showing antitumor, antidepressant, and anti-inflammatory properties and laxative effects.[5−8] The product has been widely used in the fields of food, beverage, perfume, medicine, and other industries.[9] The essential oil quality, which is affected by various factors including plant origin, method of cultivation of JFC, extraction time, parts used for extraction, extraction technology, storage method, and packaging production, commonly refers to the content of the main active components. The extraction methods of the essential oil reported in the literature commonly include solvent extraction, steam distillation, supercritical extraction method, high-pressure extraction, and ultrasound-assisted extraction.[10−14] Among them, ultrasound-assisted extraction

was studied herein due to its high efficiency, low time cost, and convenient operation. However, there were still several unresolved questions and limitations in the applications in the modern industrial process. For example, the temperature control in the extraction process: high temperatures may destroy the heat-sensitive active components in the product, but low temperatures may lead to incomplete extraction and incomplete aroma of essential oil. As a consequence, increasing the extraction rate of finger citron essential oil without destroying active components has been one of the research focuses in recent years.

"Quality by design" was a common efficient method for food and drug production extraction and process control. For achieving quality by design, the construction of a mathematical model between critical quality attributes and potential critical process parameters was emphasized, and the "point" was replaced by "space" therefore to build a design space.[15−19] In previous trials, quality by design-based methods have been successfully applied in scenarios such as pharmaceutical product development, vaccine stabilization, and RNA platform production.[20−22] On the basis of the quality by design strategy,
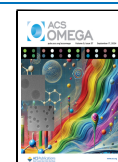
compared with the quantitative analysis by normal extraction, the quality control by normal gas chromatography (GC) analysis showed advantages including high analytical efficiency, high sensitivity, high specificity, and minor sample requirement.

Commonly, investigators try to balance the quality and quantity during the extraction process. In traditional Chinese medicine and ethnomedicine, the extract is considered an integral product to realize biological activity. Therefore, for essential oils containing functional natural products, quality control and extraction technology are significant. According to the above information, the regional distinctive industry and the medicinal field have promoted the urgent necessity of establishing the draft of quality standards for JFC essential oil. The main purpose of this work was to develop a corresponding quality by design-based method with GC analysis for optimizing the extraction technology. Here, we presented the construction of a design space where the process parameters could be flexibly selected. A definitive screening design was used to investigate the potential critical process parameters. The space design, including six statistically significant factors, was further established. The physical constant of the product was measured, and the composition of JFC essential oil was discussed. The most significant highlight of this work was constructing the optimal operating space by applying the quality by design strategy to realize advantages such as high stability and operational flexibility. The information here might be serviceable for future investigations.

2. MATERIALS AND METHODS

2.1. General Materials and Sources. JFC essential oil extraction was performed with a KQ5200DE numerical ultrasonic cleaner (Kunshan Ultrasonic Instruments Co., Ltd., Kunshan, China) and a DF-101S constant temperature magnetic stirrer (Gongyi Yuhua Instrument Co., Ltd., Gongyi, China). The apparatuses used in the ultrasonication and distillation were assembled as shown in Figure S1. The combined apparatuses helped realize the ultrasonication and distillation during JFC essential oil extraction. The analysis of the analytes was performed on a Labsolution GC-2030 system (Shimadzu, Japan) equipped with a flame ionization detector. GC-MS was performed on an Agilent Technologies 7820A GC system combined with an Agilent 5977E series GC/MSD. The measurement of the refractive index was performed on a WYA abbe refractometer (Shanghai Yi Dian Physical Optical Instrument Co., Ltd., Shanghai, China).

Sodium chloride, anhydrous sodium sulfate, and analytical-grade chemicals were purchased from Tianjin Dingshengxin Chemical Co., Ltd. (Tianjin, China). Eleven batches of *C. medica* L. var. *sarcodactylis* were collected from Jinhua Jinshoubao Biotechnology Co., Ltd. (Jinhua, Zhejiang, China) and Jinhua Academy of Agricultural Sciences. The sampling information is shown in Table 1.

2.2. Ultrasonic-Assisted Distillation Extraction of JFC Essential Oil. Initially, the basic extraction procedures of JFC essential oil were carried out. The same batch of dried fruits of *C. medica* L. var. *sarcodactylis* was ground into powder. About 100 g of the powder was sonicated with deionized water by adding sodium chloride auxiliary. After being soaked for a period of time, the mixture was extracted under ultrasonication. Then, the extractor was connected with a condenser for the next distillation extraction of essential oil

Table 1. JFC Sampling Information, Including the Source, Country of Origin, and the Batch Number

sample no.	source	country of origin	batch no.
S1	Jinhua Jinshoubao Biotechnology Co., Ltd.	Jindong District, Jinhua, Zhejiang	2020-12-22
S2	Jinhua Jinshoubao Biotechnology Co., Ltd.	Jindong District, Jinhua, Zhejiang	2020-12-23
S3	Jinhua Jinshoubao Biotechnology Co., Ltd.	Jindong District, Jinhua, Zhejiang	2020-12-24
S4	Jinhua Jinshoubao Biotechnology Co., Ltd.	Jindong District, Jinhua, Zhejiang	2020-12-25
S5	Jinhua Jinshoubao Biotechnology Co., Ltd.	Jindong District, Jinhua, Zhejiang	2020-12-26
S6	Jinhua Jinshoubao Biotechnology Co., Ltd.	Jindong District, Jinhua, Zhejiang	2020-12-29
S7	Jinhua Jinshoubao Biotechnology Co., Ltd.	Jindong District, Jinhua, Zhejiang	2020-01-05
S8	Jinhua Jinshoubao Biotechnology Co., Ltd.	Jindong District, Jinhua, Zhejiang	2020-01-09
S9	Jinhua Academy of Agricultural Sciences	Wucheng District, Jinhua, Zhejiang	2022-01-25
S10	Jinhua Academy of Agricultural Sciences	Wucheng District, Jinhua, Zhejiang	2022-01-28
S11	Jinhua Academy of Agricultural Sciences	Wucheng District, Jinhua, Zhejiang	2022-02-25

from JFC. The solution was slowly heated and slightly boiled until no more essential oil flowed out. After the extraction, the essential oil was cooled to room temperature and collected. Then, its quantity was read. An appropriate amount of anhydrous sodium sulfate was added to the product for drying.[23] The oil product was obtained after filtration. The final essential oil product extracted from JFC could be obtained after being filtered. The yield rate was calculated according to formula 1.

$$\text{yield rate (\%)} = \frac{\text{the quantity of essential oil (g)}}{\text{the quantity of raw materials (g)}} \times 100\% \tag{1}$$

2.3. Determination of Critical Quality Attribute and Potential Critical Process Parameters. The parameters were selected according to the previous reports on the extraction of essential oils.[24,25] On the basis of a single-factor test, a definitive screening design was used to investigate the uncertain process parameters, including solid−liquid ratio (X_1), auxiliary NaCl concentration (X_2), soaking temperature (X_3), soaking time (X_4), ultrasonic power (X_5), ultrasonic time (X_6), ultrasonic temperature (X_7), and distillation time (X_8). JMP software (Version 11 pro, SAS Corporation of the United States) was used for the experimental design. The regression coefficient P values of all of the parameters were calculated, and the parameters with P values lower than 0.01 were considered significant for further investigation.

2.4. Constructing the Design Space. In this section, Matlab software (R2018b, American MathWorks company) was applied for the design space development. The design space was established with a probability algorithm for error measurement through the simulation experiment. The specific programming ideas were supported by previous references.[26] The equations established for the design space involved a series of complex steps as referenced with the Monte Carlo method.[26,27] The main steps were as follows: (1) The relative standard deviation of each evaluation index was calculated. (2) Random results for all experimental conditions were generated.

Table 2. Conditions and Results of Definitive Screening Designed Experiments

group no.	solid−liquid ratio (X_1)[5]	auxiliary NaCl concentration (X_2, %)	soaking temperature (X_3, °C)	soak time (X_4, min)[6]	ultrasonic power (X_5, W)[4]	ultrasonic time (X_6, min)	ultrasonic temperature (X_7, °C)	distillation time (X_8, h)	yield (Y, %)
1	1:2	15	25	90	300	60	25	3	3.18
2	1:4	5	25	30	300	90	25	5	3.34
3	1:3	10	40	30	200	60	50	4	3.50
4	1:2	5	25	90	100	90	75	4	3.25
5	1:4	10	60	90	100	90	25	3	2.98
6	1:2	5	60	90	200	30	25	5	3.08
7	1:2	5	60	30	300	90	50	3	3.24
8	1:2	10	25	30	300	30	75	5	3.12
9	1:2	15	60	60	100	30	75	3	3.14
10	1:4	5	40	90	300	30	75	3	3.36
11	1:4	15	60	30	300	30	25	4	3.21
12	1:4	15	25	30	200	90	75	3	3.30
13	1:2	15	40	30	100	90	25	5	2.96
14	1:4	15	25	90	100	30	50	5	3.10
15	1:4	5	60	30	100	60	75	5	3.16
16	1:3	5	25	30	100	30	25	3	3.32
17	1:3	10	60	90	400	90	75	5	3.05

(3) Mathematical models were established. (4) Models to predict each evaluation index were used. (5) Probability values of attaining all preset objects were obtained. (6) All of the possible parameter combinations were calculated to get the design space. The design space consisted of an independent variable range (the yield rate of JFC essential oil) with a response value greater than 3.25%. The calculation step number was set to 5000, with a stepwise regression P value of 0.10 and the minimum probability value of reaching the standard of 0.90. The calculating step lengths of auxiliary NaCl concentration, soaking temperature, distillation time, ultrasonic power, solid−liquid ratio, and soak time were set as 0.005, 0.5, 0.05, 2, 0.01, and 1, respectively. Subsequently, by proof-reading the Akaike information criterion and the stepwise regression method, the fitting equation between evaluation indexes and process parameters was established. Then, the variance analysis was carried out.

2.5. GC Fingerprint of JFC Essential Oil. Based on the above results of the parameters, the embryonic form of the quality standard could be depicted preliminarily. (1) Extraction of JFC essential oil: In accordance with the space-optimized, optimal extraction process, 11 different batches of JFC were extracted as follows: About 50 g of the JFC powder was sonicated with 150 mL of deionized water by adding sodium chloride auxiliary with a concentration of 10%. The role of NaCl in the extraction process is to promote extraction efficiency by enhancing the polarity and density of the aqueous phase, increasing the ionic strength, and preventing water and water-soluble polar matrices in the sample from interfering with the entry of the target into the extraction solution. After being soaked at 50 °C for 60 min, the mixture was extracted under ultrasonification. The ultrasonic power was set as 200 W, the ultrasonic temperature was 50 °C, and the ultrasonic treatment time was 60 min. The conditions of process parameters were chosen to be inside the design space, but they were not exactly at the central point in consideration of both the inner connections of some parameters and the cost reduction. Then, the extractor was connected with the condenser for the next distillation extraction of essential oil from JFC. The solution was slowly heated for 4 h. After the drying step, the final essential oil was collected for the following experiments. (2) Chromatographic conditions: The separation was carried out in a DB-17 30 m × 0.32 mm × 0.25 μm GC capillary column ((50%-phenyl)-methylpolysiloxane, Waters, MA) in which nitrogen was used as a carrier gas with a flow rate of 0.5 mL/min. The conditions were as follows: inlet temperature of 65 °C for 1 min, 2 °C/min to 100 °C, 100 °C for 2 min, 50 °C/min to 250 °C, and 250 °C for 5 min. The injector temperature was held at 250 °C, and the temperature of the detector was 280 °C. The injection volume of the sample was 0.5 μL with a shunt ratio of 40:1. The solvent delay carriage gases were N_2 and H_2. (3) Preparation of test samples: 0.05 mL of the extracted JFC essential oil was accurately absorbed and put in a 2 mL volumetric flask. The absorbent medium was water. An appropriate amount of ethyl acetate was diluted and added to 2 mL scale lines as the test solution. The following steps were unfolded to further study the properties and GC fingerprint of JFC essential oil.

3. RESULTS AND DISCUSSION

3.1. JFC Essential Oil Extraction Process Optimization. As described in the experimental protocols, the basic extraction procedures of JFC essential oil were carried out and the yield rate calculation formula was determined. Subsequently, based on the general procedure, the parameters of the critical process were studied. In this work, the ultimate goal was to optimize the extraction process of the JFC essential oil; therefore, the yield rate of the essential oil (Y) was identified as the critical quality attribute. The uncertain technological parameters of the ultrasonic-assisted distillation extraction of JFC essential oil were noticed, and there were many parameters that remained to be investigated. The definitive screening design was an economic method to support the study containing a large number of factors within a small-scale experiment. After the calculation of the eight potential parameters by JMP software, a total of 17 groups of experiments were designed, as shown in Table 2.

The fitting equation between the evaluation indexes and the process parameters was established by proofreading the Akaike information criterion and the stepwise regression method.

$$Y = 3.4996 + 0.0343 \times X_1 - 0.0579 \times X_2 - 0.0221 \times X_3$$
$$+ 0.0421 \times X_4 - 0.015 \times X_5 - 0.0221 \times X_6$$
$$- 0.0507 \times X_7 - 0.012 \times X_1 X_3 + 0.0221 \times X_8$$
$$- 0.1201 \times X_3^2 - 0.0540 \times X_1 X_4 + 0.0718 \times X_4 X_6$$
$$- 0.0145 \times X_5 X_6 + 0.0374 \times X_5^2$$

Variance analysis was performed on the experimental results of the definitive screening design. The results showed that the consistency coefficient R^2 of the deterministic screening model was 0.9988 (greater than 0.75). The analysis of variance (ANOVA) P value was less than 0.0001, which indicated that the model was sufficient to fit the relationship between the yield of the critical quality attribute and process parameters investigated. Similarly, the difference between the model (R^2 = 0.9988) and the R square adjusted (R_{adj}^2 = 0.9684) further indicated the applicability of the model.

The relationship curve between the predicted and actual values of the critical quality attribute is shown in Figure 1, in
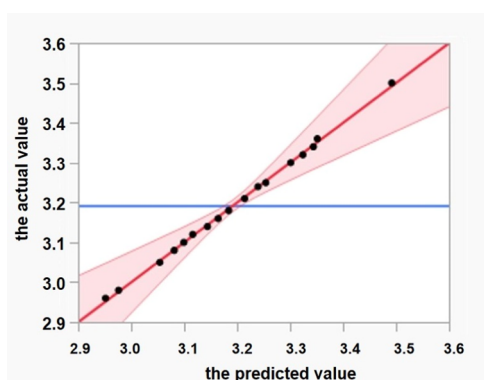

Figure 1. Relationship curve between the predicted and actual values of JFC essential oil yield. The red solid line: the fitting line between the predicted and actual values; the blue horizontal line: the mean value of the actual essential oil yield; the red shaded part: 95% confidence interval.

which the red solid line was the fitting line and the blue horizontal line was the mean value of the actual essential oil yield rate. From the simulation results, the scattered points of the actual experimental data were evenly distributed around the fitting line, and most of the scattered points were distributed within the 95% confidence interval (red shaded part). The 95% confidence interval was across the mean horizontal line, which indicated a satisfactory linear-fitting effect. The effect summary of each method parameter is presented in Table 3. The regression coefficient P values of the six method parameters, namely, auxiliary NaCl concentration (X_2), soaking temperature (X_3), distillation time (X_8), ultrasonic power (X_5), solid−liquid ratio (X_1), and soak time (X_4), were less than or equal to 0.01. According to the effect diagram, the above three process parameters had a significant impact on the critical quality attribute. Ultimately, the six potential critical process parameters were determined as auxiliary NaCl concentration (X_2), soaking temperature (X_3), distillation time (X_8), ultrasonic power (X_5), solid−liquid ratio (X_1), and soak time (X_4).

3.2. Design Space Development. As mentioned above, the design space was established with a probability algorithm

Table 3. Pareto Chart of Standardized Effects Following Response Transformation (Items were Ordered by Logworth Values)

item	logworth	P value
auxiliary NaCl concentration	2.404	0.00395
soaking temperature	2.337	0.00460
distillation time	2.290	0.00513
distillation time × distillation time	2.256	0.00555
ultrasonic power	2.131	0.00740
solid−liquid ratio × soaking temperature	2.107	0.00781
solid−liquid ratio	1.954	0.01112
soaking time × ultrasonic power	1.646	0.02260
soaking time × soaking time	1.615	0.02427
soaking time	1.584	0.02606
ultrasonic temperature	1.584	0.2606
ultrasonic time	1.265	0.05432
solid−liquid ratio × ultrasonic power	1.172	0.06726
ultrasonic time × ultrasonic time	0.135	0.73340

for error measurement through the simulation experiment. The irregular shape space design model was obtained, as shown in Figure 2. Figure 2a explores the spatial relationships of the auxiliary NaCl concentration, soaking temperature, and distillation time while the ultrasonic power was set as 200 W, the solid−liquid ratio was set as 1:3, and the soak time was set as 60 min. Figure 2b explores the spatial relationships of ultrasonic power, solid−liquid ratio, and soak time while the auxiliary NaCl concentration was set as 10%, soaking temperature set as 40 °C, and distillation time set as 4 h. The gradual change of the image from blue to yellow in Figure 2 presented the gradual increase in the probability value of reaching the standard. The selected best operating space when the probability value of reaching the standard reached 1 was as follows: the auxiliary NaCl concentration was 9−12.00%, the soak temperature was 30−50 °C, the distillation time was 3.5−4.00 h, the ultrasonic power was 200−300 W, the solid−liquid ratio was 1:3−1:3.5, and the soak time was 40−80 min. For the verification experiment, six process points in the operation space were randomly selected, among which points 1−3 were located in the yellow area of the operation space, and points 4−6 were outside the yellow area. The validation experiment arrangement with the involved parameters and results are shown in Table 4. The results showed that by setting the six potential critical process parameters to locate the points, the response value of the process points within the yellow area was higher than that outside the yellow area. Accordingly, the tested result agreed with the predicted model of the design space. Compared with the previous reports that indicated the yield rates of 1.86−3.10% for extracting *C. medica* L. species,[28−30] the design space in this work could basically guarantee a yield rate over 3.25% when the main components were similar. The design space was practical for a scale of 50 g, which was informative for connecting the laboratory trials and the pilot scale experiments.

3.3. Study of the Properties of JFC Essential Oil. (1) Determination of the relative density of JFC essential oil: The net weight of the specific pycnometer and the mass of the pycnometer filled with distilled water or liquid were successively weighed. After the calibration, the relative density of the test samples could be obtained with the measured data being inducted into formula 2. The experimental data and results are shown in Table S1.
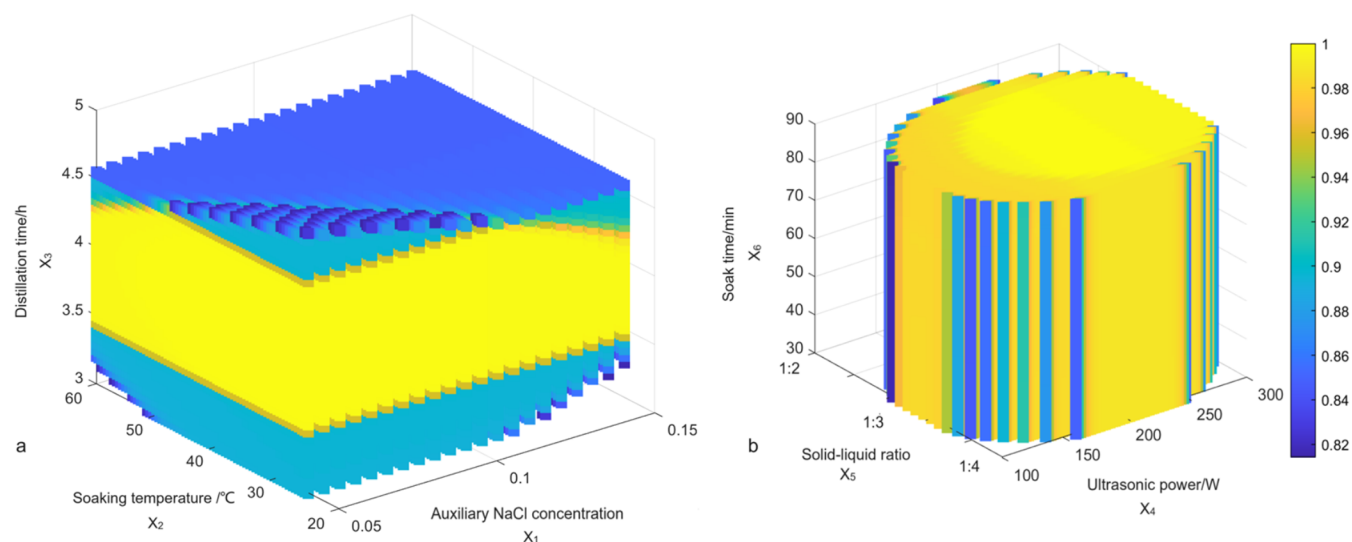
Figure 2. Design space of extraction technology of JFC essential oil. Yellow area: higher than the set response value; outside yellow area: lower than the set response value.

Table 4. Results of Validation Experiments

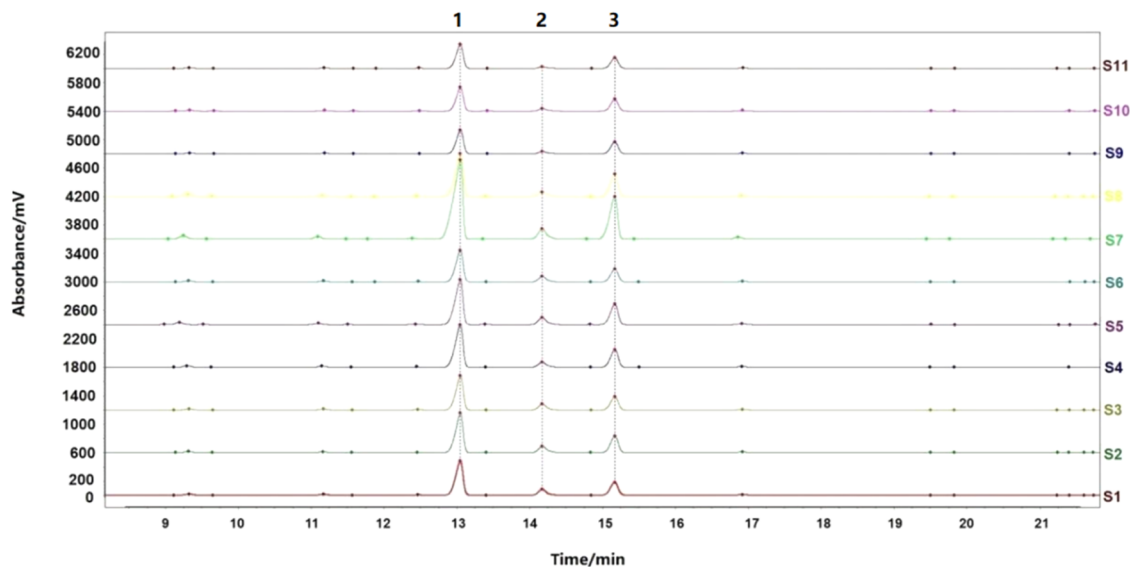| procedure order	auxiliary NaCl concentration (%)	soaking temperature (°C)	distillation time (h)	ultrasonic power (W)	solid−liquid ratio	soak time (min)	essential oil yield of JFC (%)
1	10	40	4	200	1:3	50	3.50
2	12	50	3.5	250	1:3.5	60	3.72
3	9	30	4	300	1:3	70	3.41
4	10	30	3	150	1:2	30	3.01
5	10	20	3	100	1:2.5	50	2.57
6	5	60	3	300	1:4	60	2.79

Figure 3. GC fingerprints of 11 batches of JFC essential oil. Three main characteristic peaks: peak 1, D-limonene; peak 2, γ-terpinene; peak 3, 5,7-dimethoxycoumarin.

$$\rho_{\text{liquid to be measured}} = \frac{m_3 - m_1}{m_2 - m_1} \times \rho_{\text{distilled water}} \quad (2)$$

Here, m_1 is the weight of the empty pycnometer, m_2 is the weight of the pycnometer filled with distilled water, and m_3 is the weight of the pycnometer filled with JFC essential oil. The final measured average relative density was 0.876 g/mL, which was quite close to and slightly lower than the commercial

sample density of 0.882 g/mL. This result inferred that the JFC essential oil here was almost identical to the commercial sample in density, while there was a slight tendency to contain more fatty-soluble components. (2) Determination of the relative index of JFC essential oil: The refractive index of JFC essential oil was measured according to Pharmacopoeia of The People's Republic of China, Part 1, 2020 Edition (Appendix VII) at an ambient temperature of 25 °C. Then, the interrupter
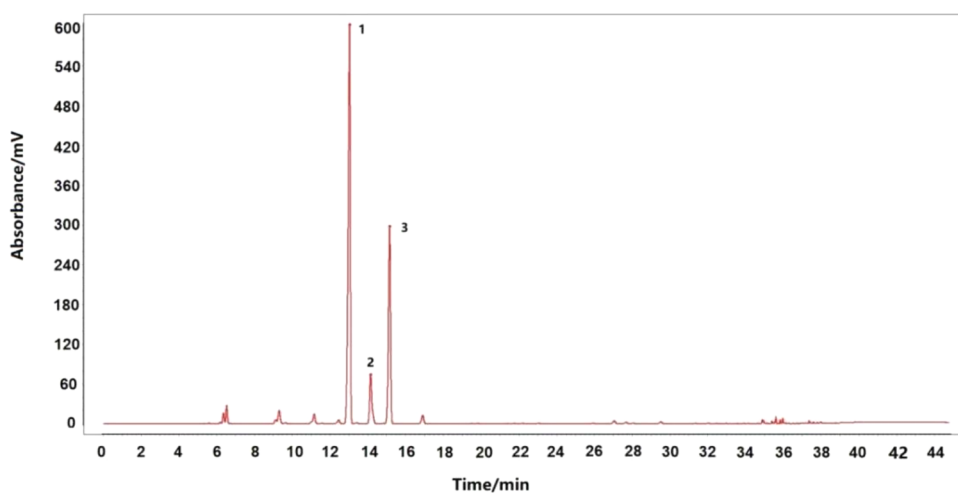
Figure 4. Reference fingerprint of JFC essential oil with characteristic peaks as a part of the quality standard. Three main characteristic peaks: peak 1, D-limonene; peak 2, γ-terpinene; peak 3, 5,7-dimethoxycoumarin.

mirror was wiped clean for the measurement of the test samples. The same measuring method was repeated three times to obtain the average refractive index of test samples. The determined results are shown in Table S2. The final measured average value of the refractive index was 1.641, which was close to and slightly higher than that of the commercial sample refractive index of 1.637. The result was consistent with that of the density test. (3) Determination of the pH value of JFC essential oil: The pH values of different batches of JFC essential oil were determined by using accurate pH test paper at 25 °C. The results are shown in Table S3. The pH values of the tested batches were in the range of 4.5−5.0, while a majority of them were 5.0, which was consistent with that of the commercial sample. Above all, the verification of density, refractive index, and pH value indicated that the JFC essential oil was almost the same as the standard commercial JFC oil.

3.4. Analysis of the GC Fingerprint of JFC Essential Oil. (1) Gas chromatographic analysis of different batches of samples: The essential oil extracted from 11 batches of JFC was selected to prepare the test samples according to the method mentioned above. An equivalent testing sample was injected for GC detection. The peak area of each peak was integrated, and the proportion of nonshared peaks was less than 8%, as shown in Table S4. In these samples, the essential oil yields of JFC were all over 3.45%, slightly lower than the value in the design space (3.54%) but much higher than that at the unoptimized condition (2.79%). Therefore, using optimal conditions led to a high essential oil yield of JFC. (2) Similarity analysis of different batch samples and establishment of fingerprint: The GC analysis results were imported into the similarity evaluation system for the chromatographic finger-print of TCM (Version 2004 A). The fingerprint map of 11 batches of JFC essential oil is shown in Figure 3, in which there were three main characteristic peaks. The similarity of different batches of products was calculated, as shown in Table S5. The results showed that the similarity of the GC analysis results of 11 batches of JFC essential oil was above 0.99, which indicated that the difference in the chemical composition between each batch of JFC essential oil was not obvious. In the fingerprint mapping system, the gas chromatogram of Sample 1 was taken as the reference and the width of the time window was set as 30 min. After the multiple corrections of Marker peak

matching, the control map under the common pattern of JFC essential oil was obtained, as shown in Figure 3. Additionally, the reference fingerprint was generated from the GC fingerprints in our research, which showed the stability of the approach and the guiding significance for future investigations (Figure 4 and Table S5).

3.5. Identification of Main Peaks by GC-MS. The three main characteristic peaks were then identified by GC-MS by an Agilent Technologies 7820A GC system combined with an Agilent 5977E series GC/MSD using the same condition as described for the GC assay. We used the reference standard compounds with an external reference method by using the same condition. Accordingly, the three main characteristic peaks were identified as follows: peak 1: D-limonene, peak 2: γ-terpinene, and peak 3: 5,7-dimethoxycoumarin. Moreover, by investigating the GC fingerprints and the main peaks, the composition of the main components of JFC essential oil was relatively clear. The most important components were D-limonene, γ-terpinene, and 5,7-dimethoxycoumarin. Other components including pinene and phellandrene, as referenced, showed shorter retention times, while the less-amount species including geranial and neral showed longer retention times.[31]

4. CONCLUSIONS

The extraction efficiency and quality control of essential oil extracted from traditional Chinese medicine is an important step in the production process, which is of great significance to the stability and reproducibility of drug efficacy. The JFC essential oil was taken as the research object in this project. Based on the theory of quality by design, the ultrasonic-assisted distillation extraction process was comprehensively and systematically investigated. The yield rate of JFC essential oil was defined as the critical quality attribute. The definitive screening design was first applied to the investigation of potential critical process parameters. The six most potential critical process parameters in the production were auxiliary NaCl concentration, soaking temperature, distillation time, ultrasonic power, solid−liquid ratio, and soak time. Therefore, these six factors should be strictly controlled in actual industrial production or laboratory extraction. The most important step in quality by design is the establishment of the design space. The multiunit operation design space with three potential critical process parameters was established, and

the operation space throughout the whole process improved the operational flexibility of the production process. The optimal operating space was as follows: the auxiliary NaCl concentration was 9−12.00%, the soak temperature was 30−50 °C, the distillation time was 3.5−4.00 h, the ultrasonic power was 200−300 W, the solid−liquid ratio was 1:3−1:3.5, and the soak time was 40−80 min. The selection within this region could help determine the best manufacturing conditions to fulfill the quantity standard. With the method in this work, the essential oil yields of JFC were all over 3.45%, significantly higher than the previously reported value of 3.01‰.[32,33] The application of the quality by design strategy realized various advantages, including high stability and operational flexibility. The design space and preliminary quality standards established in this paper could be extended to the actual scale production and thus could be conducive to standardizing the production and quality control of products. Meanwhile, the above workmanship could improve the rationality, specificity, and efficiency of product exploitation procedures.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03065.

Relative density measurement results; refractive index measurement results; pH value determination results; proportion of noncommon peaks; and similarity evaluation results (PDF)

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Notes

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