ISSN 2616-5910 Vol. 5, Issue 1: 158-167, DOI: 10.25236/AJALS.2024.050121

Preparation process and component analysis of natural high-purity capsaicin

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Abstract: *In order to explore the efficient preparation process of natural high-purity capsaicin, Yunnan devil pepper was selected as the starting material for the study. Through systematic solvent screening and orthogonal experimental design, the extraction conditions of capsaicin were optimized. The optimal extraction process was determined to use 75% ethanol as the solvent, extract at 65 ℃ for 2 hours, and set the solid-liquid ratio to 1:4. For the crude extract obtained by the optimal extraction process, a graded impurity removal strategy was adopted, followed by steps such as ethyl acetate water extraction, steam distillation, activated carbon adsorption, and n-hexane crystallization to improve the purity of capsaicin. Subsequently, the high-purity capsaicin prepared was subjected to chromatographic separation to obtain a single component. Detailed composition analysis and structural identification of the separated monomers were performed using infrared spectroscopy and nuclear magnetic resonance spectroscopy. The experimental results show that the preparation process can effectively extract and purify capsaicin, and the resulting product is in powder form with a yield of 0.83% and a purity of 95.15%. The preparation process proposed in this study is not only easy to operate, but also has good feasibility and practicality, providing scientific basis and technical reference for the large-scale production of natural high-purity capsaicin. The successful implementation of this process is expected to promote the widespread application of capsaicin in the fields of medicine, food, and chemical engineering.*

Keywords: *high-purity capsaicin; Preparation process; Graded impurity removal; Component analysis; Deep processing of chili peppers*

1. Introduction

Capsaicin is a key chemical substance that endows chili peppers with spicy characteristics. Its structural features include vanilla groups and medium to long chain fatty amides, belonging to the category of alkaloids. Up to now, more than 20 capsaicin compounds have been identified from chili peppers, with typical representatives including capsaicin, dihydrocapsaicin, dihydrocapsaicin, high capsaicin, and high dihydrocapsaicin. As food additives, capsaicin like substances can significantly enhance the spiciness and irritability of food, facilitate precise control of spiciness, and are easy to use. Currently, the application scope of capsaicin has broken through the limitations of the food industry and has widely penetrated into multiple fields such as medicine, military, and chemical engineering. Given the various biological effects exhibited by capsaicin in human health, such as promoting gastric mucosal repair, preventing gastric ulcers, accelerating blood circulation, enhancing immunity, and anti-cancer activity, its application in the fields of food and medicine is becoming increasingly widespread.

At present, the extraction technology of natural capsaicin has undergone development and has gradually moved from traditional solvent extraction methods to a new stage of high efficiency and diversification. Based on the physicochemical properties and similar solubility principle of capsaicin, extraction using solvents such as alcohols, acetone, and ethyl acetate has become a conventional method. On this basis, by adding surfactants to improve extraction efficiency, or adjusting the extraction solvent to alkaline to utilize the phenolic hydroxyl acidic characteristics of capsaicin, the extraction rate can be further increased. In addition to single-phase solvent extraction, two-phase or multi-phase solvent systems, such as polyethylene glycol ethyl and methyl imidazolate acetate biphasic systems, as well as three-phase salt precipitation methods, have also been widely explored. However,

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although these methods are effective, they increase the complexity of post-processing, make it difficult to reuse reagents, and limit production volume. With the advancement of technology, external auxiliary measures such as microwave, ultrasound, enzyme treatment, and supercritical extraction have been introduced, significantly promoting the dissolution of capsaicin, improving extraction efficiency, and reducing solvent usage. However, these methods still face challenges such as complex equipment and high operating costs.

In response to the current situation where the application of natural capsaicin is mainly limited to chili oil (chili oil resin) and the production process of high-purity products is scarce and costly, especially the challenge of traditional column separation methods being difficult to achieve large-scale production, this study aims to explore an efficient and low-cost high-purity capsaicin production pathway. By selecting high spiciness Yunnan devil pepper as the raw material and conducting in-depth research on the extraction and purification technology of capsaicin, the aim is to develop a simple, economically reasonable, and industrialized production standard capsaicin preparation process. This process aims to effectively utilize raw materials, improve production efficiency, and ensure the acquisition of high-purity capsaicin products, thereby providing strong support for the wider application of capsaicin in various fields.

2. Materials and Methods

2.1 Materials and reagents

The chili raw materials used in this study were purchased from the Jiangdongpo area of Mangshi, Yunnan. After drying, they were finely ground using a grinder and screened through a 60 mesh sieve to ensure uniform particle size. The chemical reagents used include 95% ethanol, ethyl acetate, n-hexane, and petroleum ether (boiling point range 60-90 ℃), all of which are of analytical grade and provided by Tianjin Kemio Reagent Co., Ltd. In addition, two chromatographic pure reagents, acetonitrile and methanol, were also purchased from Tianjin Kemio Reagent Co., Ltd. The deionized water required during the experiment is prepared in the laboratory to ensure pure water quality and meet the experimental requirements.

2.2 Main instruments and equipment

This study used multiple high-precision and high-performance instruments and equipment to support the experimental process. Among them, the MS7-H550 Pro magnetic stirrer is provided by Dalong Xingchuang Experimental Instrument Co., Ltd. and is used for mixing and stirring operations in experiments. The BSA223S electronic analytical balance, sourced from Saitoris Biotechnology Co., Ltd., ensures accurate measurement of the mass of experimental substances. The RE-52AA rotary evaporator, produced by Shanghai Yarong Scientific Instrument Co., Ltd., effectively achieves rapid evaporation and recovery of solvents. MP200 medium pressure preparation liquid chromatograph, provided by Tianjin Bonaijer Technology Co., Ltd., is used for efficient separation and purification of samples. The U3000 high-performance liquid chromatograph, manufactured by Thermo Fisher Scientific Co., Ltd., achieves precise analysis of sample components. In addition, an AVANCE I 400 MHz nuclear magnetic resonance spectrometer, produced by Bruker AG in Switzerland, was used to investigate the structural characteristics of the sample molecules in depth.

2.3 Method

2.3.1 Capsaicin Extraction

2.3.1.1 Selection of solvents for capsaicin extraction

To determine the optimal solvent for extracting capsaicin, the experimental design was as follows: Accurately weigh 100g of chili powder and divide it into 9 portions. Add water, 95% ethanol, and ethyl acetate to each portion in a solid-liquid ratio of 1:4 (g/mL). Each sample was soaked for 0.5 hours and then refluxed for 2 hours under stirring conditions. After completion, solid-liquid separation was achieved through filtration, and the resulting filtrate was subjected to solvent removal using a rotary evaporator at 60 ℃ to obtain capsaicin extract. The extraction process of each solvent was carried out in parallel three times to ensure the accuracy and reliability of the results, and the average of the three experiments was taken as the final result.

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2.3.1.2 Optimization selection of capsaicin extraction process

After determining the optimal extraction solvent, in order to further screen for the best extraction conditions, this study adopted an L9 (34) orthogonal experimental design. This design focuses on the selected solvent (which has been determined to be optimal in previous experiments), considering three key factors: extraction temperature, extraction time, and solid-liquid ratio, and setting three different levels for each factor. The specific factors and level designs are shown in Table 1. Through this orthogonal experiment, the aim is to systematically explore the effects of various factors on the extraction efficiency of capsaicin, in order to determine the optimal combination of process parameters and achieve efficient extraction of capsaicin.

Table 1: L_9 (3^4) Orthogonal Experiment Factor Level Table

code	factor	level		
		1	2	3
A	time/h	1	2	4
B	solid-liquid ratio/(g/mL)	1:2	1:4	1:6
C	temperature/℃	60	70	80
D	ethanol concentration/%	50%	75%	95%

2.3.2 Purification of Capsaicin

2.3.2.1 Removal of water-soluble substances

To effectively remove water-soluble impurities from the crude extract of capsaicin, the following steps were taken in the experiment: dissolve the crude extract of capsaicin in ethyl acetate at a ratio of 1:4 (g/mL), and then add an equal volume of deionized water for extraction. After thorough mixing, layering is achieved by utilizing the difference in solubility between the aqueous and organic phases of the substance, followed by discarding the aqueous phase containing water-soluble impurities. This process is repeated twice to ensure that water-soluble impurities are fully removed. [1]Finally, the ethyl acetate layer was concentrated using a rotary evaporator at 60 ℃ until a paste like substance was formed, which resulted in the removal of water-soluble impurities from capsaicin.

2.3.2.2 Volatile Oil Removal Process

To further purify capsaicin, it is necessary to remove its volatile oil components. The experiment adopts the following steps: transfer the oil like capsaicin obtained in the above steps to a distillation flask, and add deionized water at a ratio of 1:10 (g/mL). Subsequently, steam distillation was carried out until the distillate lost its pungent odor, indicating that the volatile oil had been effectively removed. Next, add an equal volume of ethyl acetate to the upper oily substance for extraction to separate any residual volatile oil components. After stratification, collect the upper layer liquid and concentrate it under vacuum at 60 ℃ until ethyl acetate is completely removed, finally obtaining capsaicin in the form of a dark brown paste after volatile oil removal.

2.3.2.3 Crystallization process

To obtain high-purity capsaicin, the capsaicin treated with water-soluble substance removal and volatile oil removal is purified by crystallization. The specific operation is as follows: transfer the processed capsaicin to a distillation flask and add n-hexane in a ratio of 1:2 (g/mL). Heat and stir under reflux for 10 minutes to ensure that the paste like capsaicin is fully dissolved in n-hexane. Subsequently, activated carbon was added for decolorization treatment. After removing activated carbon by hot filtration, cool the filtrate to room temperature and refrigerate at 4 ℃ for 12 hours to promote the crystallization and precipitation of capsaicin. Finally, the crystals were collected through filtration, and the resulting filter cake was a powder of high-purity capsaicin.[2]

2.3.3 Purity analysis method for capsaicin

In HPLC analysis, accurately weigh 1g of powdered capsaicin and place it in a 100mL volumetric flask. Then, dilute it to the mark with a uniformly mixed methanol tetrahydrofuran solution to obtain a sample solution with a concentration of 10mg/mL. This solution is filtered through a 0.45 μ m microporous membrane and used as a sample solution for later use. Chromatographic analysis was performed using a C18 column (specification 4.6mm × 250mm, particle size 5 μ m), with methanol water solution (volume ratio 65:35) as the mobile phase, maintained at a flow rate of 1mL/min, and detection wavelength set at 280nm. The experimental parameters strictly follow the GB28314-2012 "Food Additive Chili Oil Resin" standard to ensure accurate calculation of capsaicin purity. The purity of capsaicin is calculated according to the following formula.[3]

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$$w = \frac{c \times V}{1000m}$$

In the formula: C - the capsaicin content found from the standard curve, in μ g/ml; V-constant volume of the sample, in mL; 1000- Quality conversion factor; The mass of the sample is measured in grams.

2.3.4 Analysis of Capsaicin Components

2.3.4.1 Liquid phase separation for capsaicin preparation

Accurately weigh 5g of high-purity capsaicin powder, dissolve it thoroughly in 10mL of methanol, and use this solution as the sample for loading. Using the liquid sample loading method, inject the sample into a Flash column filled with C18 bonded silica gel. Subsequently, the sample was separated using a semi preparative liquid chromatography system. During the separation process, the ratio of the mobile phase was set to a volume ratio of methanol to water of 60:40, the flow rate was constant at 8mL/min, and the detection wavelength was set to 280nm. Through the above separation conditions, 2.76g of capsaicin monomer and 1.46g of dihydrocapsaicin monomer were successfully isolated from the sample, achieving effective separation and purification of capsaicin compounds.[4]

2.3.4.2 Infrared Spectral Analysis of Capsaicin

Take 2mg of each of the capsaicin monomer and dihydrocapsaicin monomer obtained by chromatographic separation, mix them thoroughly with 100mg of spectroscopically pure potassium bromide, and then compress them into tablets to prepare samples suitable for infrared spectroscopy analysis. Subsequently, the prepared samples were placed in an infrared chromatograph for analysis and determination. During the measurement process, set the scanning wavenumber range of the instrument to 4000-400 cm ^ -1 to ensure coverage of all possible infrared absorption peaks in the sample. The scanning resolution is set to 2 cm ^ -1 to improve the resolution and accuracy of the spectrum. At the same time, set the scanning frequency to 32 times, and further improve the signal-to-noise ratio and reliability of the data by averaging multiple scans.

2.3.4.3 Capsaicin Nuclear Magnetic Spectrum Analysis

Take approximately 10mg of each of the capsaicin monomer and dihydrocapsaicin monomer obtained by liquid chromatography separation, and transfer them into dedicated nuclear magnetic tubes. Subsequently, add 0.5mL of deuterated chloroform to each nuclear magnetic tube and ensure that the sample is fully dissolved in deuterated chloroform through shaking operation. After complete dissolution, use a 400MHz nuclear magnetic resonance spectrometer to perform nuclear magnetic resonance analysis on the sample. During the analysis process, 1H NMR (hydrogen nuclear magnetic resonance) and 13C NMR (carbon nuclear magnetic resonance) spectra of the sample were collected separately to obtain chemical environment information of hydrogen and carbon atoms in the sample molecules, and then infer the molecular structural characteristics of the sample.[5]

3. Results and Analysis

3.1 Research on the Extraction Process of Capsaicin

3.1.1 Comparison of extraction rates of capsaicin using different solvents

Figure 1 shows the efficiency comparison of extracting capsaicin from chili powder using three different solvents: water, ethanol, and ethyl acetate. The results showed that ethanol had the highest extraction rate, followed by water, and ethyl acetate had the lowest. During the initial 0.5-2-hour period of the extraction process, the extraction rate of capsaicin significantly increases with time, but after more than 2 hours, the change in extraction rate with time is no longer significant.

This phenomenon may be attributed to the dissolution mechanism of capsaicin from chili cells. The dissolution of capsaicin requires the solvent to effectively penetrate and diffuse into chili cells, thereby dissolving capsaicin and forming a concentration gradient inside and outside the cells. High concentration solutions tend to diffuse from the inside of cells to the outside, while low concentration solvents continuously enter the inside of cells, and this process repeats until dynamic equilibrium is reached. In the early stage of extraction, due to the large concentration difference between inside and outside the cell, the extraction rate is relatively fast. However, as the concentration difference gradually decreases, the extraction rate also slows down accordingly.[6]

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In terms of solvent permeability, water performs the best, followed by ethanol, and ethyl acetate performs the worst. This may be the main reason for the relatively low extraction rate of ethyl acetate. Although water has good permeability, its solubility in the target component (i.e. capsaicin) is relatively low, making it not an ideal extraction solvent. In contrast, ethanol not only has better permeability, but also can dissolve capsaicin with higher solubility, thus demonstrating comprehensive advantages in extraction efficiency and target component recovery rate. Therefore, ethanol is considered a suitable solvent for extracting capsaicin.

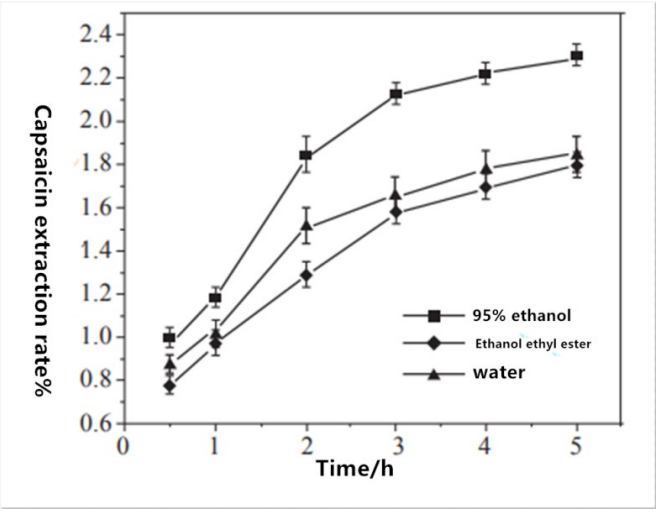

Figure 1: Comparison of extraction rates of capsaicin by different solvents

3.1.2 Orthogonal experimental design and results

Based on the results of solvent screening experiments, it has been determined that ethanol has the highest extraction efficiency for capsaicin, while water produces more paste like substances, but the capsaicin content is relatively low. In order to effectively extract other beneficial components from chili while improving the extraction rate of capsaicin, this study designed an orthogonal experiment to comprehensively investigate the specific effects of four key factors, namely extraction time (A), solid-liquid ratio (B), extraction temperature (C), and ethanol concentration (D), on the efficiency of capsaicin extraction. The experiment used 100g of chili powder per serving as raw material and extracted it by adding a mixed solvent of ethanol and water. [7]By precisely controlling the level changes of each factor and combining the experimental results shown in Tables 2 and 3, the contribution of each factor to the extraction efficiency can be systematically analyzed, and the optimal combination of extraction process parameters can be determined. This orthogonal experimental design not only helps to deepen the understanding of the impact mechanism of various factors on the extraction process, but also provides a scientific basis for optimizing the extraction process of capsaicin and other useful components.

Table 2: Orthogonal experimental results of capsaicin extraction

Test number	A	B	C	D	Ointment yield 1%	Total Capsaicin Content/ (g/100g)
1	1	1	1	1	7.83	1.69
2	1	2	2	2	8.62	2.34
3	1	3	3	3	9.20	2.18
4	2	1	2	3	8.37	2.27
S	2	2	3	1	8.34	2.42
6	2	3	1	2	8.59	2.37
7	3	1	3	2	8.16	2.51
8	3	2	1	3	8.34	1.78
9	3	3	2	1	8.45	2.03
kl	2.07	2.16	1.95	2.05		
k2	2.35	2.18	2.21	2.41		
k3	2.11	2.19	2.37	2.08		
R	0.28	0.03	0.42	0.36		

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Table 3: Analysis of Variance

Variance comes source	SS (Sum of squares)	f	MS (mean square)	F	P	significance
A	0.1425	2	0.0712	0.8650	0.4570	*
B	0.0021	2	0.0010	0.0125	0.9875	
C	0.2749	2	0.1374	1.6689	0.2479	*
D	0.2394	2	0.1197	1.4536	0.2894	*
error	0.6588	8				

By analyzing the range of the orthogonal experimental results, as shown in Table 2, the degree of influence of each factor on the extraction rate of capsaicin can be clearly determined. According to the analysis of R value, the extraction temperature has the most significant impact on the extraction rate of capsaicin, and the degree of influence of each factor from high to low is as follows: extraction temperature, ethanol concentration, extraction time, and solid-liquid ratio. Further analysis shows that the optimal process conditions for extracting capsaicin are ethanol concentration of 75%, extraction temperature of 65 ℃, extraction time of 2 hours, and a solid-liquid ratio of 1:4.

Meanwhile, Table 3 of the analysis of variance provides more in-depth statistical evidence, indicating that extraction temperature and ethanol concentration have a significant impact on capsaicin content, extraction time also has a significant effect, while the effect of the solid-liquid ratio is not significant. This result may be related to the selection range of the solid-liquid ratio in the experiment. In this experiment, the minimum material to liquid ratio selected was 1:2 (g: ml), under which a large amount of capsaicin could already be extracted. Therefore, when increasing the extraction solvent further, although it can improve the extraction rate to a certain extent, the change in extraction rate is relatively small and no longer a key factor affecting the extraction efficiency. Through orthogonal experimental design and data analysis, this study not only clarified the specific effects of various factors on the extraction rate of capsaicin, but also successfully optimized the extraction process conditions, providing strong technical support for the industrial production of capsaicin.[8]

The experimental results showed that using a 75% ethanol aqueous solution as the extraction solvent can effectively dissolve capsaicin and other components. As the temperature increases, the solubility of the solvent in the target component increases, thereby promoting the extraction of capsaicin. However, considering that the amide structure contained in the target component may decompose at high temperatures, choosing 65 ℃ as the extraction temperature is more suitable. Under these conditions, combined with a parameter setting of extraction time of 2 hours and a material to liquid ratio of 1:4, not only can capsaicin be fully extracted, but it can also effectively save extraction time and subsequent extraction liquid concentration costs. This conclusion provides strong data support for the efficient extraction and industrial application of capsaicin.

3.2 Research on Purification Process of Capsaicin

Based on the physical and chemical properties of capsaicin (trans-8-methyl-6-nonenamide) and drawing on existing research on dried chili components, this study systematically carried out the separation and removal of components other than capsaicin in chili crude extracts. The experimental process includes the removal of water-soluble impurities (using ethyl acetate water extraction method), the removal of volatile components (using steam distillation technology), the removal of pigment components (through activated carbon adsorption treatment), and the final purification step (using n-hexane for crystallization). This series of processes aims to effectively remove impurities with different properties, thereby ensuring the purity of capsaicin. Throughout the entire process, high-performance liquid chromatography technology is used to continuously monitor the extracted liquid after each process, in order to accurately track its changing trend and objectively evaluate the treatment effect. As shown in Figures 2 to 5, through this series of carefully designed steps, not only were various impurities successfully removed, but the purity of capsaicin was also significantly improved, laying a solid foundation for the in-depth research and application development of capsaicin.

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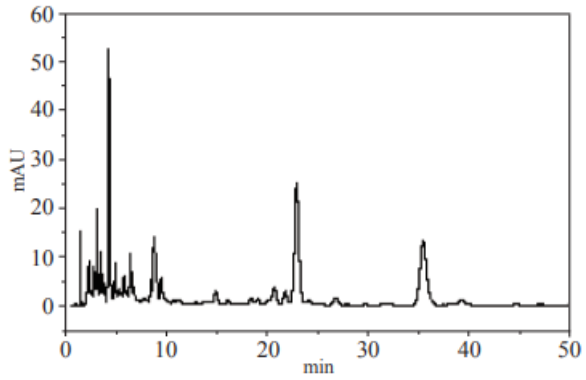

Figure 2: Liquid chromatogram of crude capsaicin extract

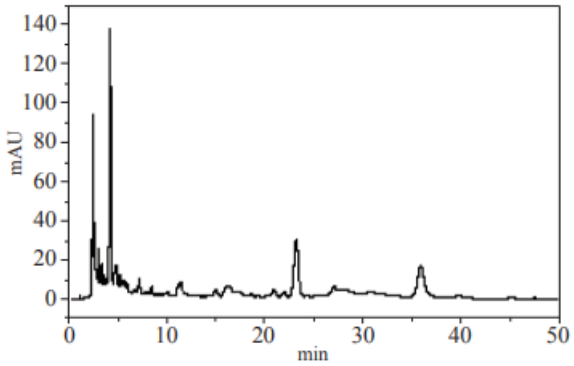

Figure 3: Liquid chromatogram of capsaicin extract after removal of water-soluble impurities

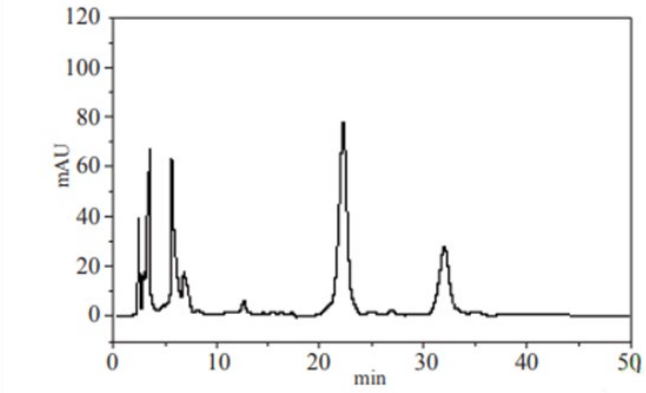

Figure 4: Liquid chromatogram of capsaicin extract after steam distillation

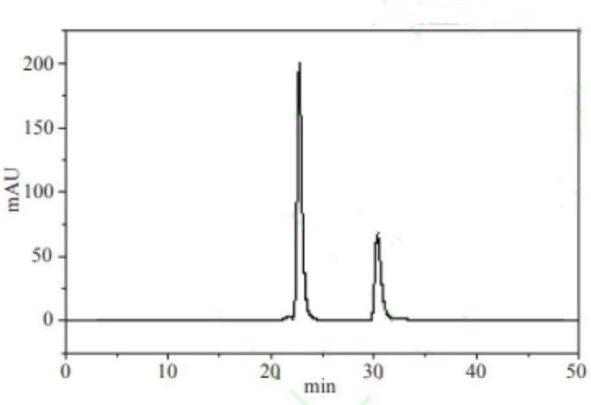

Figure 5: Liquid chromatogram of capsaicin extract after crystallization with n-hexane

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In the liquid chromatograms shown in Figures 2 to 5, it can be observed that the chromatographic peak at approximately 22 minutes corresponds to capsaicin, while the chromatographic peak at 35 minutes corresponds to dihydrocapsaicin. The multiple peaks stacked within the 0-10 minute interval represent impurities with higher polarity, while the peak after 40 minutes corresponds to impurities with lower polarity. The variation patterns of these chromatograms clearly indicate that the capsaicin separation method designed in this study can effectively remove polar water-soluble components and components with low polarity from the extract.

In addition, we attempted to use n-hexane (or petroleum ether) to heat and dissolve the crude extract of capsaicin, followed by decolorization treatment using activated carbon. We then filtered while hot and allowed it to stand at 4 ℃ for 12 hours to achieve crystallization. However, no crystallization of solid capsaicin was observed in the experimental results. Based on this phenomenon, it is speculated that the presence of impurities may interfere with the crystallization process of capsaicin. These impurities may increase the viscosity of the solution system, reduce its fluidity, and hinder the agglomeration of crystal particles, making it difficult to form crystal clusters. At this point, the solution system exhibits insoluble viscous substances adhering to the bottom or wall of the container. At the same time, the oil components in the extract may also affect the precipitation of capsaicin, as these components have a certain solubility in capsaicin, thereby interfering with its effective separation from the solution. These findings provide valuable references for further optimizing the extraction and purification process of capsaicin.

Effective removal of impurities is crucial in the preparation of solid capsaicin. The use of liquid chromatography at a wavelength of 280nm to detect the treated liquid helps optimize the separation and purification process, thereby obtaining high-quality brown powdered capsaicin products. The liquid chromatography detection results are shown in Figure 7. It is worth noting that after the crystallization step, combining and concentrating the filtrate, and then repeating the separation and purification process, a small amount of capsaicin can still be recovered from the filtrate. This measure not only improves the recovery rate of capsaicin, but also gives the final concentrated filtrate new application value, which can be used as chili oil resin. This discovery not only optimizes the preparation process of capsaicin, but also expands the application scope of the product, providing strong support for the industrial production and comprehensive utilization of capsaicin.

3.3 Analysis of Capsaicin Components

3.3.1 Component analysis of high-purity capsaicin

By using preparative liquid chromatography technology to separate high-purity capsaicin, two monomeric compounds can be successfully obtained. Subsequently, these two compounds were subjected to recrystallization treatment using n-hexane, and the products were dried in a vacuum drying oven at 40 ℃ for 2 hours. After this series of processing, the obtained product was subjected to infrared spectroscopy (IR) and nuclear magnetic resonance (NMR) detection to further confirm its chemical structure and purity. This process provides a reliable method for the efficient separation and purification of capsaicin monomers, and also lays a solid foundation for subsequent structural analysis and application research.

Compound 1: After analysis, the compound appears as a light yellow powder with distinct infrared spectral data characteristics, including O-H and N-H stretching vibration absorption, methyl and methylene CH stretching vibration absorption peaks, carbonyl and C-N bending vibration absorption of amides, and carbon carbon double bond and benzene ring skeleton vibration absorption. The nuclear magnetic resonance hydrogen spectrum and carbon spectrum data are also consistent with the data of capsaicin in the literature, specifically manifested as signal peaks at specific chemical shifts consistent with the literature. Based on the above information, it can be concluded that the compound is capsaicin, and its liquid chromatography retention time is consistent with literature reports, located at 23 minutes, as shown in Figure 6.

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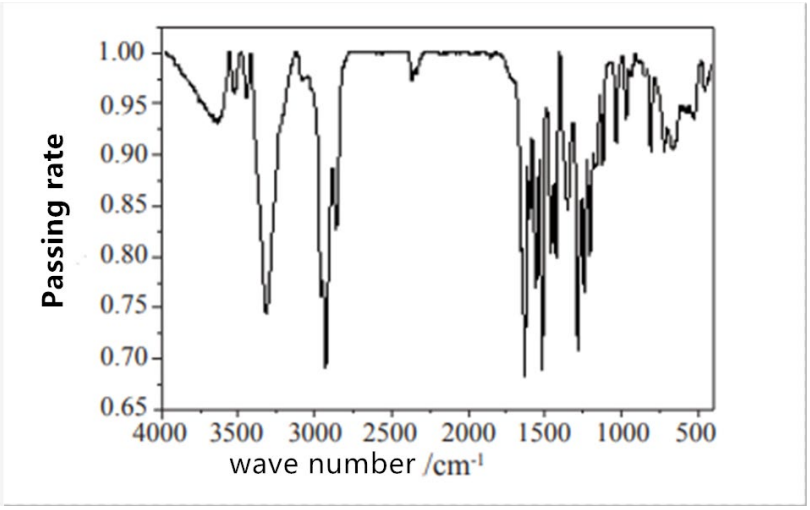

Figure 6: Infrared spectrum of capsaicin

Compound 2: This compound appears as a light yellow powder with significant infrared spectral data characteristics, including O-H and N-H stretching vibration absorption, methyl and methylene C-H stretching vibration absorption peaks, carbonyl and C-N-H bending vibration absorption of amides, and benzene ring skeleton vibration absorption. The nuclear magnetic resonance hydrogen spectrum and carbon spectrum data are also consistent with the research data of Goll J and Frey A, specifically manifested as signal peaks at specific chemical shifts consistent with literature. Based on the above information, the compound can be identified as dihydrocapsaicin, and its liquid chromatography retention time is consistent with literature reports, located at 32 minutes.

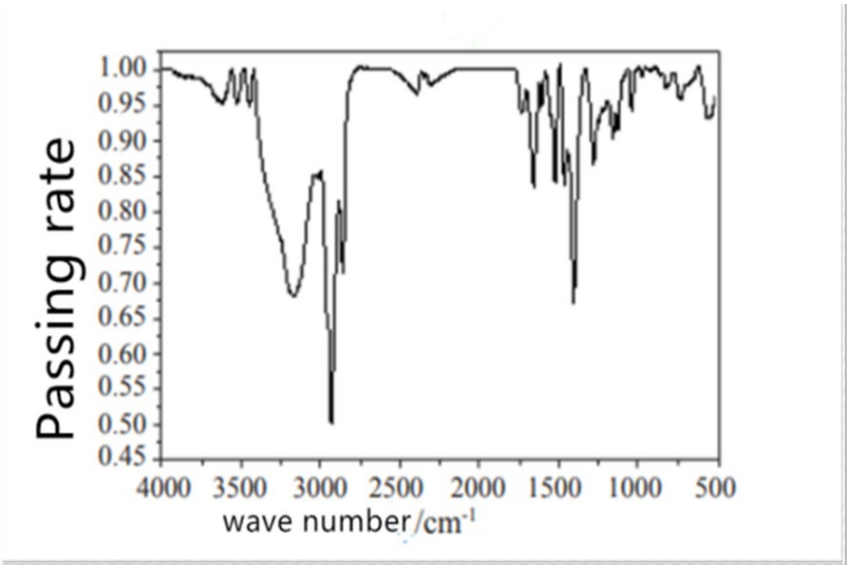

Figure 7: Infrared spectrum of dihydrocapsaicin

3.3.2 Content of various components of high-purity powdered capsaicin

The food additive chili oil resin currently sold in the market generally has a capsaicin content of no more than 20% and appears as a dark red viscous liquid. The high-purity capsaicin prepared by this research process presents a brownish yellow powder solid form, and the total content of capsaicin substances exceeds 95%. Among them, the main component capsaicin accounts for about 63%, and dihydrocapsaicin accounts for about 33%. This proportion is consistent with the relevant reports of the Ministry of Agriculture and Rural Affairs on the classification and terminology of chili products. In order to provide reference for large-scale production, Table 4 details the mass of crude extract that can be obtained per 100g of chili pepper using this process, the mass of high-purity capsaicin obtained after separation and purification, and the specific mass of two main monomers, capsaicin and dihydrocapsaicin. These data not only demonstrate the efficiency of the research process, but also provide important basis for subsequent industrial production and quality control.

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Table 4: Relevant parameters of crude capsaicin extract and high-purity capsaicin

	trait	mass yield g/100g	Capsaicin g/100g	Dihydrochili pepper g/100g
crude extract	Paste like substance	9.40±0,50g	1.10±0.05	0.67±0.06
High purity capsaicin	powder	0.50±0.10g	0.33±0.04	0.17±0.03

4. Conclusion

This study determined the optimal process conditions for extracting capsaicin through systematic exploration, using 75% ethanol as the solvent, extracting at 65 ℃ for 2 hours, and setting the solid-liquid ratio to 1:4. The extracted capsaicin substances under these conditions have a concentration of over 13%, fully meeting the requirements of the national standard GB28314-2012 "Food Additive Chili Oil Resin" for capsaicin content, laying a solid foundation for subsequent separation and purification steps. Furthermore, through refining processes such as ethyl acetate and water two-phase extraction, steam distillation, activated carbon decolorization, and n-hexane crystallization, powdered capsaicin with a capsaicin content of over 95% was successfully obtained. Its purity meets the standards for pharmaceutical capsaicin specified in the United States Pharmacopeia USP35-NF30 (2017). It is worth noting that the solvents used throughout the entire process are of low toxicity and low boiling point type. After concentration treatment, they can be completely removed without causing pollution to the environment and the final product. The remaining product after separation and purification, with its rich capsaicin content, can be flexibly added to various chili products according to actual needs, thus achieving comprehensive and efficient utilization of chili resources.

Acknowledgement

This research was supported by the Scientific Research Projects of Department of Education, ZheJiang Province in 2020, hosted by Li ShuangXi, from XingZhi College, ZheJiang Normal University.

References

[1] Dong Xinrong, Liu Zhonghua, Li Benxiang, etc. Preparation and purification of three types of capsaicin monomers by preparative high-performance liquid chromatography [J]. Chromatography, 2008, 26 (3): 366-369

[2] Ren Zhaoyan, Zhang Hui, Liu Yushen. Purification of Chili Essence and HPLC Determination of Capsaicin [J]. Chinese Food Additives, 2002 (1): 75-76, 86

[3] Dong Xinrong, Wang Jin, Liu Zhonghua, etc. Research on Fluorescence Analysis Method of Capsaicin [J]. Research and Development of Natural Products, 2007, 19 (1): 135-137

[4] Song Aijie, Niu Liangfeng, Zhang Chao, etc. Preparation methods and structural confirmation of capsaicin and dihydrocapsaicin [J]. Shandong Chemical Industry, 2019,48 (1): 22-24

[5] Randall S ,James C ,Kimberly G , et al.161 Evaluation of intra-articular CNTX-4975 (highly purified, synthetic trans-capsaicin) for knee OA pain in patients with radiographic presence of OA in the opposite knee[J].Rheumatology,2019,58(S3)

[6] Changxia L ,Ruican L ,Peng Z , et al.Separation of capsaicin from capsaicinoids by macroporous resin adsorption chromatography.[J].Journal of separation science,2015,38(23):4141-5.

[7] A N F ,G A M ,J M D , et al. Effect of highly purified capsaicin on articular cartilage and rotator cuff tendon healing: An in vivo rabbit study.[J].Journal of orthopaedic research : official publication of the Orthopaedic Research Society,2015,33(12):1854-60.

[8] E. D ,R. P ,T. S , et al. Efficacy and safety of a single intraoperative administration of highly purified capsaicin formulation for management of postoperative pain associated with bunionectomy: a randomized phase 3 trial[J].The Journal of Pain,2009,10(4S):S53-S53.