RESEARCH ARTICLE

Comparative study and optimization of nicotine extraction efficiency from waste tobacco by ethanol-ultrasound-supercritical CO_2 extraction

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Converting waste smoke into valuable compounds can effectively reduce resource waste. This study aimed to compare and optimize three methods that extracted nicotine from waste tobacco including ethanol extraction, the ultrasonic-assisted extraction using ethanol as a solvent, and supercritical carbon dioxide extraction with pyrolysis. By adjusting and optimizing process parameters, the optimal nicotine extraction conditions of the three methods were determined, among which the traditional ethanol extraction used 95% (v/v) ethanol with a material-liquid ratio of 1:10 at 70°C for 180 min, the ultrasonic-assisted extraction using ethanol applied the ultrasonic power of 150 W at a constant temperature of 70°C for 60 min for three times, and the supercritical CO_2 extraction with pyrolysis pyrolyzed 1 kg of treated waste cigarette end at 450℃ and then passed into the supercritical CO_2 equipment for three times by the entraining agent with a concentration of 70% at 55℃, 30 MPa, for 2 h. The results showed that the amounts of nicotine extracted by the three methods were 1.23, 2.13, and 43.17 mg/g, respectively. The advantage of combined pyrolytic supercritical CO_2 extraction method demonstrated a significantly higher extraction rate compared with ethanol extraction and ethanol ultrasound combined extraction with the extraction rate being increased by 20-fold and 35-fold, respectively. In addition, the method of supercritical carbon dioxide extraction with pyrolysis had higher applicability and purification effect. It was a feasible solution to efficiently extract purified nicotine from waste tobacco. These results provided an important reference for the effective use of waste tobacco resources and the industrial application of nicotine.

Keywords: waste cigarette end; ethanol; ultrasound; supercritical CO_2; extraction rate.

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Introduction

Tobacco organic waste refers to organic solid waste such as the waste of tobacco leaves, ash, cigarette ends, and cigarette stems during the production and processing of the tobacco industry. Unreasonable disposal methods will not only result in a waste of resources, but also exacerbate environmental pollution because nicotine in tobacco waste is toxic to soil microorganisms. The reutilization of tobacco waste can both reduce pollution and recover energy, thus promoting green, low-carbon, and sustainable development of China's tobacco industry [1]. Waste tobacco ends and waste tobacco pyrolysis oils are byproducts of tobacco manufacturing. As the tobacco consumption increases, the number of these wastes also grows. These wastes are rich in bioactive compounds, among which nicotine is a component with significant pharmacological activity and potential for industrial applications.

Nicotine, which accounts for 0.5-6% of the dry weight of tobacco, is an important alkaloid [2]. It is often present in tobacco plants as organic acid salts such as citric acid and malic acid [3, 4]. With an excitatory and paralyzing effect on the nervous system, nicotine can be used in the pharmaceutical industry to produce drugs that treat snake and insect bites, itchy skin, swelling, and pain. Nicotine can be oxidized to obtain nicotinic acid, which is widely used in the food sector, the chemical sector, and medical sector [5]. With the development of China's chemical, agricultural, and pharmaceutical industries, high-purity nicotine is in high demand in the market. Therefore, the study of the extraction method of nicotine has significant scientific value and potential applications.

Traditional methods to extract nicotine from tobacco leaves include ion exchange [6, 7], water vapor distillation [8, 9], extraction [10, 11], and Soxhlet's extraction method [12]. The ion-exchange method has a lengthy operating time, a low product yield, and will easily produce toxic resin. Thus, this method cannot be promoted for industrial application and needs further investigation. Water vapor distillation is simple and easy to operate, but the processing equipment is too large. With low efficiency and low nicotine purity, this method cannot meet the market demand for high-purity nicotine, so it is seldom applied in the industry. The extraction method has a certain industrial foundation, in which the organic solvent can choose ether, chloroform, vinyl chloride hydrocarbon, and so on. Recent studies on nicotine extraction often investigate how to improve processing speed, increase extraction rate, save energy and make the extraction process more environmentally friendly. The frequently used nicotine extraction methods include solvent extraction, ultrasonic/microwave-assisted extraction, and supercritical CO_2 fluid extraction. Solvent extraction method uses ether, chloroform, and other low-boiling solvents to extract nicotine in the aqueous phase. The obtained substance is then distilled to remove the organic solvent and extract nicotine. The basic process is as: dried tobacco → extraction → filtration → organic solvent extraction → extract → separation → nicotine [13, 14]. To prevent emulsification in organic solvent extraction, Lu *et al*. purified the aqueous phase using distillation before extraction and found that distillation with salt could increase the extraction rate by 10% [15]. Liu *et al*. extracted nicotine from tobacco leaves using the extraction method [16]. The optimal synthesis conditions were 50℃ for 30 min before ether extraction for five times. The highest yield of nicotine extraction was 7.34%. Although the solvent extraction method is simple and frequently used in industrial production, it is not efficient enough, because it consumes a large amount of solvent and takes a long time. Gradually, this method is replaced by ultrasonic/microwave-assisted method. Ultrasonic-assisted extraction method is an ultrasound-based new technology. It can quickly dissolve the active ingredients in the material into the solvent to get a mixture of extracts. After the extracts are separated, refined, and purified, the target chemical composition can be finally achieved. Ultrasound-assisted technology can extract nicotine in a rapid and efficient way as ultrasound produces strong vibration. Moreover, its cavitation effect, stirring, and other effects accelerate the extraction of nicotine from the solvent [17]. Compared with the traditional extraction method, the ultrasound-assisted extraction significantly reduces the extraction time. However, its extraction rate has not surpassed 90%. Zhu *et al*. achieved a 92.2% nicotine extraction rate through the enzymatic hydrolysis of the spent secondary tobacco leaves. Before ultrasonic extraction, the temperature was controlled to be constant for 1.5 h [18]. Li *et al*. used ultrasonic waves to help the simultaneous extraction of chlorogenic acid, brassinolide, cannabinol, and nicotine from tobacco [19]. 80%, 95%, 80% ethanol solution or a combination of 80%, 80%, 95% ethanol solution were used as the extraction solvents. The results showed that the extraction rates of the four target substances were all above 99.75%. The extraction rate of ultrasonic-assisted method is still unsatisfactory. However, if the solvents were changed for multiple ultrasonic extractions, a higher extraction rate can be achieved and more active substances in tobacco can be extracted at the same time. Supercritical fluid extraction uses a fluid as an extractant to separate and extract a specific component from the liquid or solid. The temperature and pressure of this technique are both higher than the critical values required to reach the thermodynamic state of the fluid. CO_2 fluid can replace toxic, hazardous, flammable, and volatile organic solvents to extract the target components under mild critical conditions (T_c = 31°C; $P_c = 7.48 \times 10^6$ Pa). So supercritical CO_2 fluid extraction is currently the most studied extraction method. After extracting tobacco wastes with water for 24 hours, Rincón *et al*. conducted supercritical CO_2 fluid extraction for 3 hours and found that, when the extraction pressure was 15-30 MPa and extraction temperature was 50-70°C, the method was the most effective with a maximum extraction rate of 99.43% [20]. Dong *et al*. used supercritical CO_2 fluid extraction and decompression distillation to extract nicotine from tobacco [11]. Although the extraction rate was not as high as that of Rincón's method, the purity of nicotine was more than 98%, which satisfied the standard of first-grade products. The supercritical CO_2 fluid extraction method can obtain nicotine with high purity and the reagents it used are environmentally friendly. However, it requires a higher extraction pressure and a longer extraction time than ultrasonic or microwave-assisted methods. Moreover, the equipment is too expensive for manufacturers to expand production. Nonetheless, it can be used in laboratory to prepare standard nicotine. Pyrolysis methods have also been used to improve nicotine yields [21]. To increase nicotine yields and purity, it is better to combine multiple methods because much more can be accomplished as part of a portfolio than a single method working alone.

This study compared the traditional process and the combined extraction process using waste tobacco as raw material. The effects of different methods on nicotine extraction rate were analyzed, and different experimental conditions were set up to explore the optimal nicotine extraction rate measured by the three processing routes. The traditional ethanol extraction route was used to explore the extraction rate of nicotine by comparing three factors of feed-to-liquid ratios, extraction temperatures, and extraction times. The ultrasound-assisted extraction process was investigated to optimize traditional extraction process. Three parameters including ultrasonic power, extraction time, and the number of extractions were focused. The combination of supercritical carbon dioxide ($scCO_2$) extraction with pyrolysis were also studied. Different pyrolysis temperatures were set to find the optimal nicotine precipitation rate in the products of pyrolysis. To find the optimal extraction rate, the supercritical CO_2 extraction method was used again under the optimal conditions of pyrolysis. During the pyrolysis, the concentration of the extraction agent, extraction temperature, extraction pressure, extraction time, and the number of extraction times were appropriately adjusted. The efficient recovery and extraction of nicotine from waste tobacco can maximize the use of resources, thereby reducing the cost of raw materials and help enterprises to achieve sustainable development.

Materials and methods

Sample pretreatment and GC-MS analysis

Tobacco waste, which was mostly crushed tobacco leaves from Yunnan Province of China, was provided by Hongta Tobacco (Group) Co., Ltd, Yuxi, Yunnan, China). The sample was stored at 22°C with a humidity of 60%. The tobacco waste was cut into pieces and dried at 70℃ for 60 minutes before crushed with an air flow high-speed universal pulverizer (China Tuoyan Instrument Co., Ltd., Cangzhou, Hebei, China) to obtain a yellow uniform powder, which was then sealed and stored at 4℃. The gas chromatography mass spectrometry (GC-MS) (Yunnan Provincial Analytical Testing Center, Kunmin, Yunnan, China) was used to analyze the relative content of nicotine in the processed tobacco waste. The proportion of nicotine in the

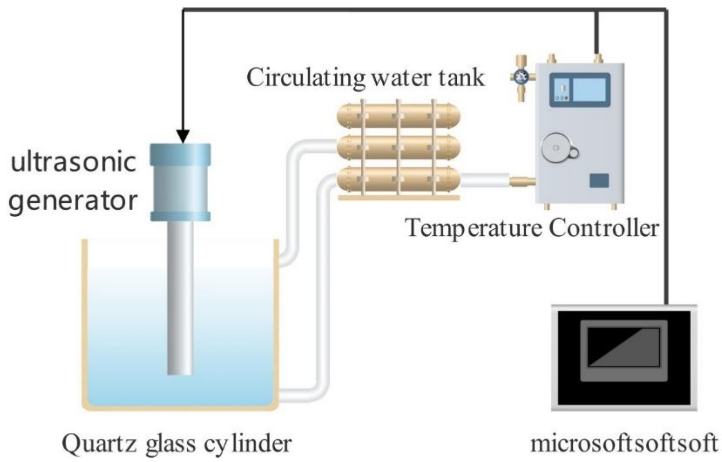

Figure 1. Diagram of ethanol-ultrasonic combined extraction device.

waste tobacco was measured to calculate the extraction rate of nicotine. 10 g of processed tobacco powder was used to test the nicotine content,

Ethanol extraction

The traditional ethanol extraction method was performed by mixing 20 g of treated tobacco powder with 95% (v/v) ethanol at the material-liquid ratio of 1:10 and incubated at 70℃ for 3 hours. The process ensured the full contact of tobacco leaves with ethanol in the soaking solution. The extraction solution was then collected, and ethanol was subsequently removed from the extract using an SB-1100 rotary evaporator (Tianjin Tester Instrument Co., Ltd., Tianjin, China) to obtain the concentrated extract. The extract containing nicotine was filtered and placed at 4°C for sealed storage.

Ethanol ultrasonic joint extraction

The ethanol-ultrasound-assisted extraction was performed using ethanol-ultrasonic combined extraction device with adjustable ultrasonic power of 0 – 200 W, frequency of 28/40 KHz provided by Shenzhen Futian Company (Shenzhen, Guangdong, China) (Figure 1). The treated tobacco samples were mixed with 95% (v/v) ethanol at 1:10 ratio and deposited in the ultrasound chamber at 70°C. The ultrasonic power was adjusted from 30 to 200 W at a gradient of 30 W. The ultrasonic frequency was set at 40 KHz, and the ultrasonic duration was adjusted from 20 to 60 min at a gradient of 20 min. After the ultrasonic extraction, a filter was used to separate the solid residue to obtain the extract. After removing the ethanol through the evaporation and concentration method, the concentrated extract was obtained. The final extract was stored in a sealed container at 4°C.

Combined pyrolysis supercritical CO_2 extraction

The pyrolysis oil collection system used two-stage condensation. The high-purity nitrogen with 99.99% purity was used as the carrier gas in the pyrolysis with a flow rate of 1,000 mL/min. 1,000 g of the treated soot samples were weighed and placed in a quartz reactor. Nitrogen was passed for a period before the experiment to remove the air in the device, and a resistance furnace was used to increase the temperature with a heating rate of 5℃/min. After the temperature changed from room temperature to the experimental temperature, the soot was left at the final temperature for 120 min to ensure sufficient pyrolysis. To explore the effect of temperature on nicotine, the initial pyrolysis temperature was set at 200℃. The temperature gradient ranged from 50℃ to 600℃ with a total of 8 temperature groups. After reaching the setting temperature, each group stayed for 120 min. The pyrolysis solution was collected and placed at 4℃ for storage. The supercritical CO_2 passed through the extraction device (Nova

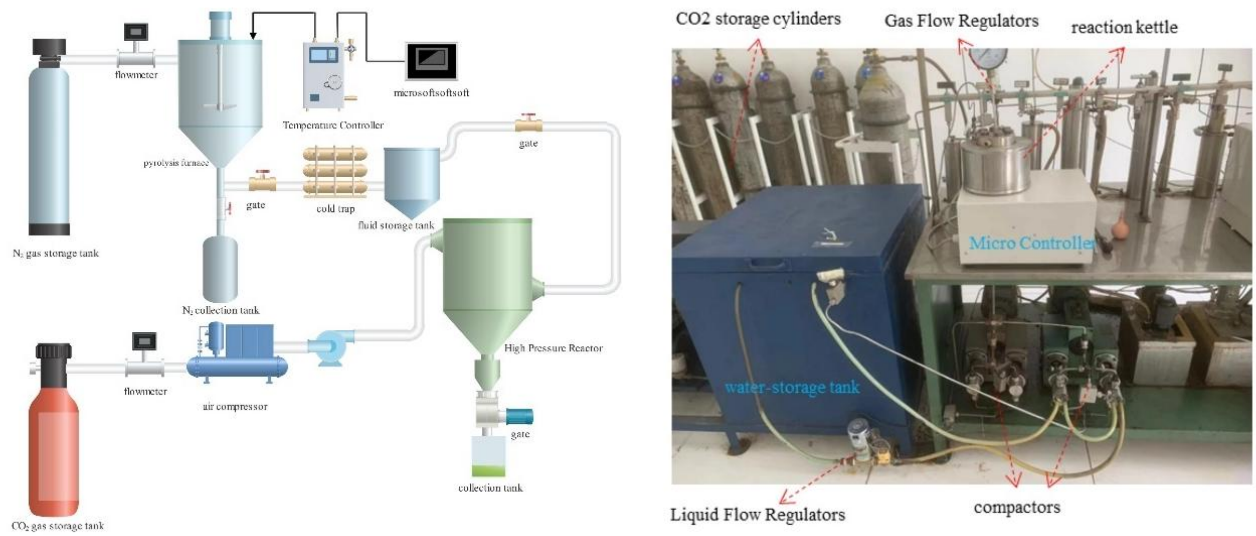

Figure 2. Experimental setup for combined pyrolysis supercritical CO_2 extraction.

Werke AG, Illnau-Effretikon, Switzerland) into the waste tobacco samples for supercritical CO_2 extraction. During this process, supercritical CO_2 under high pressure and high temperature was present in both liquid and gaseous states, which could more efficiently extract nicotine. To study the effect of supercritical CO_2 on nicotine extraction, the refrigerated 20 mL pyrolysis solution was placed in the supercritical CO_2 equipment, and the temperature and pressure values were adjusted. Two temperature values of 315℃ and 325℃ were included, and the pressure was incremented from 22 MPa to 30 MPa at 2 MPa increase rate. The extraction time was set to 30 min, 60 min, and 90 min, respectively. The dosage was 2 mL, and the optimal extraction conditions were obtained by adjusting the experimental parameters. CO_2 was evaporated by depressurization to obtain the nicotine-containing extracts, which were sealed and stored at 4℃.

Calculation of nicotine extraction rate

The nicotine extraction rate was calculated below.

$$\text{Niacin extraction rate} = \frac{M \times X}{M_0 \times X_0} \times 100\% \qquad (1)$$

where M was the mass of concentrate in the leachate (g). X was nicotine content in concentrate (g). M_0 was the mass of raw material (g). X_0 was nicotine content in raw material (g).

Results and discussion

Reliability of the method

The measured content of nicotine was 8.77 mg/g, accounting for 43.85% of the powdered tobacco content. To ensure the accuracy of each experiment, the accuracy of the measured nicotine data was compared with the curve of the nicotine standard (Figure 3). The results showed that the detected nicotine curves were consistent with the nicotine standard data. Therefore, the detected data of the nicotine content of the discarded end of the cigarettes were reliable.

Analysis of ethanol extraction results

The traditional method of nicotine extraction using ethanol takes advantages of different solubility phases between ethanol and nicotine. Nicotine is a fundamental compound in tobacco. Ethanol is an organic solvent and can physically or chemically interact with nicotine, causing it to dissolve in ethanol. This method made the

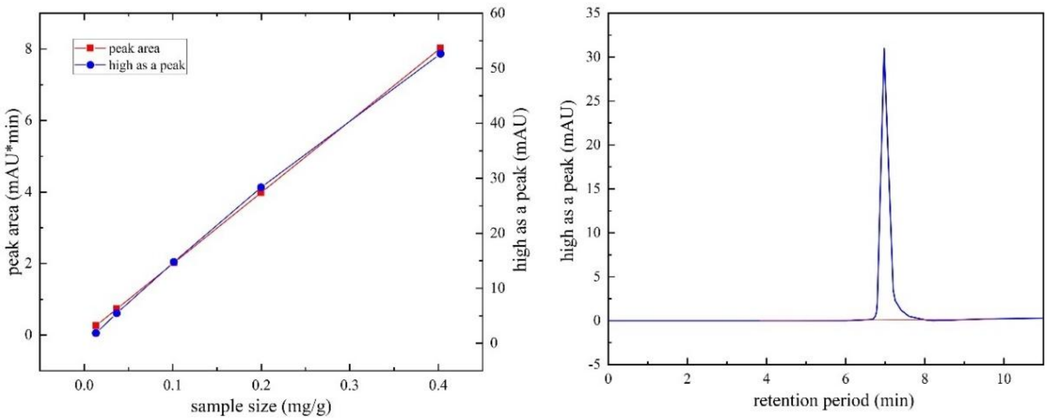

Figure 3. Calibration and chromatographic profiles of nicotine samples.

tobacco leaves and ethanol fully contacted and immersed. When the nicotine precipitation time was 6.03 minutes, the peak height of 6.20 mAU appeared. The peak area was 0.94 mAU·min, and the sample was detected containing 1.23 mg/g of nicotine. The nicotine extraction rate Y was calculated as 44.05% according to the equation (1).

Analysis of combined ethanol ultrasonic extraction

The principle of ethanol-ultrasound-assisted extraction is based on the mechanical, thermal, physical, and chemical effects of ultrasound and the solubilizing effects of ethanol as a solvent. When ultrasound waves propagate through the solvent, an acoustic phenomenon is produced, which leads to physical and chemical changes in the liquid. The violent fluid dynamics effect produced by ultrasound can disrupt the cellular structure and release the nicotine inside the cell. In addition, ultrasound also accelerates nicotine's transfer from solid sample to solution. In this way, the nicotine extraction is facilitated. The mechanical energy of ultrasound is converted into thermal energy, resulting in increased molecular vibrations in the liquid, which increases the extraction rate. Moreover, ultrasound can change the chemical properties of the extraction solvent, increasing the polarity and solubility of the ethanol solvent. More interaction force between the ethanol solvent and nicotine increases the solubility and extraction efficiency. The results showed that the extraction rate of nicotine increased rapidly with the extraction temperature (Figure 4a). When the temperature reached 60°C, the growth of the extraction rate leveled off. Therefore, 70°C was selected as the extraction temperature. With the extension of ultrasonic extraction time, the extraction rate increased faster (Figure 4b). When the extraction time exceeded 60 min, the extraction rate gradually stabilized. If the extraction time continued to be extended, it would waste energy. Thus, the optimal extraction time of nicotine was selected as 60 min. The extraction rate increased gradually with more ultrasonic power and reached the maximum value when the power was 120 W (Figure 4c). Therefore, 120 W was selected as the optimal ultrasonic power. The extraction rate increased significantly with the increase in the number of extractions. The extraction rate almost stopped growing when the number of extractions reached 4 times (Figure 2d). Based on the calculation, the extraction rate was 84.03% after two extractions and 89.01% after 3 extractions. After considering the production cost and efficiency, 3 extractions were selected. The analytical results showed that nicotine's precipitation time after ultrasonic extraction was 6.11 min. After the peak height of 11.39 mAU and peak area of 1.64 mAU·min appeared, the sample contained 2.16 mg/g of nicotine. The highest concentration of the extraction rate after the three ultrasonic extractions was 89.1%. The optimal extraction

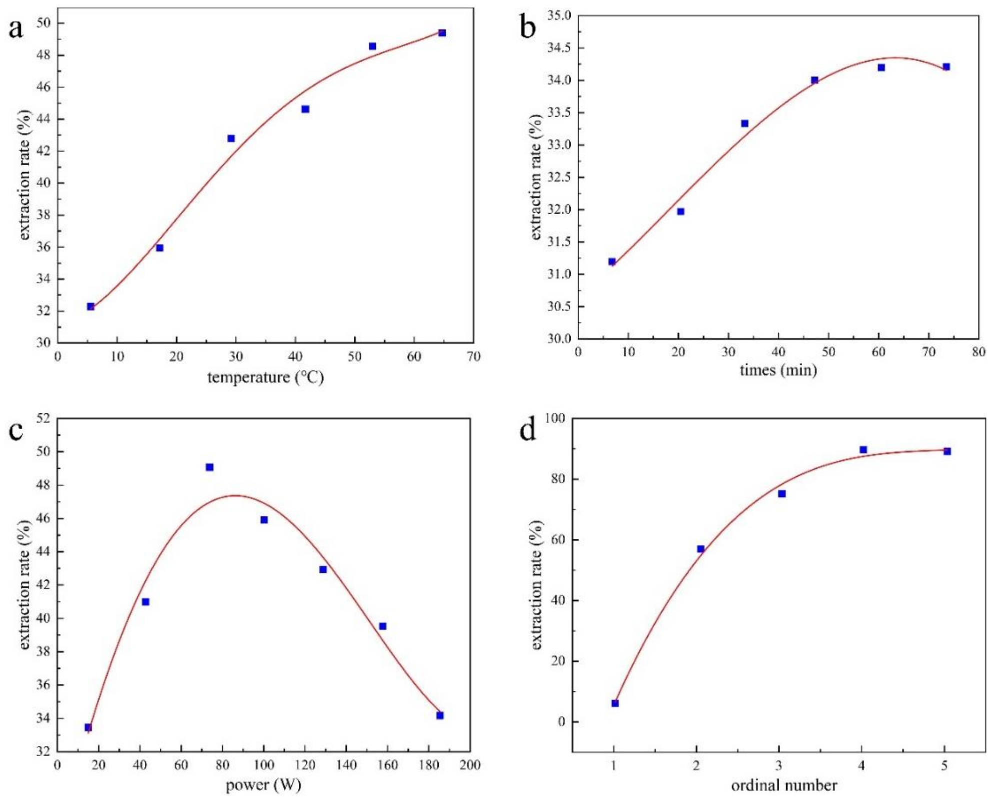

Figure 4. The effects of ultrasonic extraction temperature (a), ultrasonic extraction time (b), ultrasonic power (c), and number of extractions on nicotine extraction rate (d).

temperature was 70℃, and the volume fraction of ethanol was 95%.

Analysis of combined pyrolysis supercritical CO_2 extraction

Pyrolysis supercritical CO_2 combined extraction is a new method of extracting nicotine. The pyrolysis of treated cigarette end at high temperature can remove other compounds. The supercritical CO_2 is a special state of matter. Under high pressure and high temperature, CO_2 can be changed into a supercritical state with its density and solvency transformed to liquids. However, its diffusion properties are like gases. This special state of matter makes supercritical CO_2 an ideal solvent for the effective extraction and separation of natural products. In the supercritical state, the CO_2 solubility is controlled by temperature and pressure and CO_2 can be adjusted to selectively dissolve the target substances. Therefore, the effective combination of these two methods promoted the production of higher-purity nicotine. When the pyrolysis temperature was at 450℃, nicotine's relative content was the highest, up to 44% (Figure 5). The pyrolysis experiments also identified the relative contents of nicotine at the temperature of 450℃ during pyrolysis (Figure 6). The supercritical experiments were designed to explore the optimal extraction conditions (Tables 1 to 3). The results showed that the optimal condition parameters were a carrier concentration of 70%, extraction temperature of 325°C, extraction pressure of 30 MPa, extraction time of 2 hours, which resulted in a nicotine extraction rate of 97%.

Comparison of three extraction methods

To compare the effects of the three methods on nicotine's extraction, the mechanisms of three methods on nicotine extraction were analyzed. The traditional method of extracting nicotine with ethanol is based on the different solubility phases between ethanol and nicotine. Nicotine is

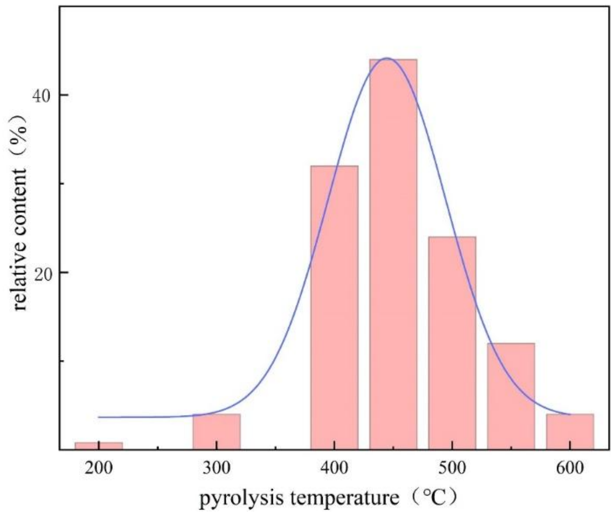

Figure 5. The plot of nicotine yields as a function of temperature.

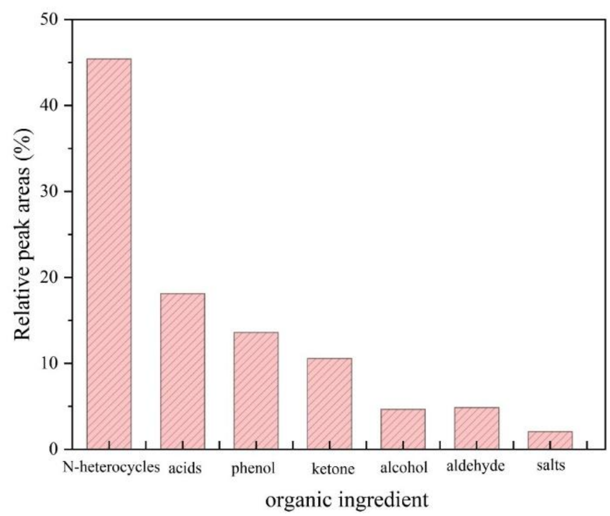

Figure 6. Distribution of organic fractions and relative peak areas of pyrolysis oil from tobacco waste at 450°C.

a basic compound found in tobacco. Ethanol is an organic solvent that can physically or chemically interact with nicotine to dissolve it. In the ethanol-ultrasound-assisted extraction, the acoustic vibrations generated by ultrasonic waves in the liquid can cause microscopic eddy currents in the liquid and break the liquid film, thereby increasing substances' rate of mass transfer, which accelerates the dissolution of compounds such as nicotine from plant material into ethanol. In addition, ultrasound can cause the rupture of cell walls, thus accelerating the release of compounds. Ethanol can be used as an effective solvent for nicotine extraction. Therefore, the ethanol-ultrasound-assisted extraction of nicotine can promote nicotine extraction through the mechanical action of ultrasonic waves and the solvent action of ethanol. Supercritical CO_2 plays an important role in the combined extraction of nicotine. CO_2 is both a solvent and a reactant. The potential mechanisms could be the occurring of mass transfer phenomenon after the supercritical CO_2 encountering the nicotine in the raw material

Table 1. Effect of extraction pressure on nicotine yield.

Extraction pressure (MPa)	Extraction temperature (°C)	Extraction rate (%)
22	315	75
24	315	80
26	315	84
30	315	96
22	325	82
24	325	91
26	325	94
30	325	98

Table 2. Effect of extraction time on the yield of nicotine.

Extraction time (h)	Extraction temperature (°C)	Extraction Pressure (MPa)	Extraction rate (%)
0.5	315	26	56
1	315	26	67
1.5	315	26	77
2	315	26	83
0.5	325	30	61
1	325	30	77
1.5	325	30	82
2	325	30	98

Table 3. Effect of extraction temperature on the yield of nicotine.

Extraction temperature (°C)	Extraction pressure (MPa)	Extraction rate (%)
305	26	56
315	26	80
325	26	91
330	26	96
305	30	62
315	30	83
325	30	94
330	30	97

and the gradually dissolving of nicotine into the supercritical CO_2. By controlling the temperature and pressure, the density and solvency of supercritical CO_2 could be adjusted, thus affecting the solubility and extraction effect. The extraction was realized by transferring nicotine from the raw material to supercritical CO_2 under a certain temperature and pressure. After the pyrolysis of supercritical CO_2, nicotine could be separated and collected to obtain a higher-purity product. The results showed that the ethanol-ultrasound-assisted extraction method introduced ultrasound to the traditional method and could effectively enhance the extraction rate of nicotine. The combined supercritical CO_2 extraction with pyrolysis of tobacco at high temperatures could remove other compounds and resulted in increased and higher purity nicotine (Figure 7). Organic solvents in the extraction are more environmentally friendly. Compared with the traditional extraction method, the extraction rate increased by two times. The traditional ethanol extraction method was simple and low-cost. However, the purity of

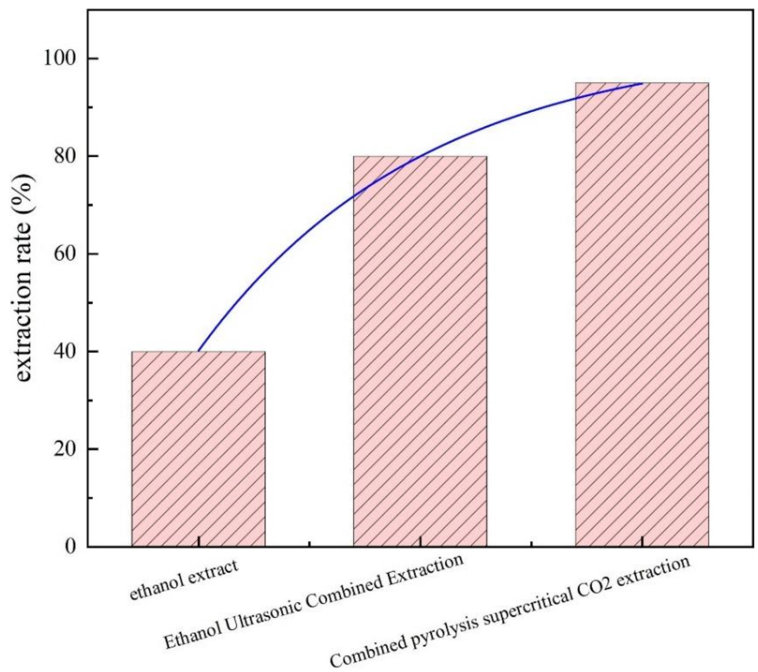

Figure 7. Comparison of extraction rates of different processes.

the yielded nicotine was not high. Ethanol-ultrasound-assisted extraction had better extraction efficiency than the traditional method with a shorter extraction time and a higher yield. However, its overall extraction rate was less than 90%. Pyrolysis supercritical CO_2 extraction was efficient and did not require organic solvents, so there was no pollution to the environment, which could reach an extraction rate of over 90%. However, the process was more complex than the traditional method and the ethanol-ultrasound-assisted method. Therefore, this method was suitable for research in high-end areas. The selection of suitable methods should be decided based on the actual needs and experimental conditions.

Conclusion

Three methods that extracted nicotine from waste tobacco were compared, which included the traditional method of ethanol extraction, the ethanol-ultrasound-assisted extraction, and pyrolysis-supercritical CO_2 co-extraction. The ethanol-ultrasound-assisted extraction had better extraction effect and easy operation procedure. Compared with the traditional extraction method, ultrasound-assisted extraction process had shorter extraction time. The nicotine content obtained was two times more than that of the traditional extraction process, but the extraction rate was still less than 90%. This method was suitable for extracting nicotine from the cigarette end with the optimal conditions of 120 W of ultrasonic power, 40 Khz of ultrasonic frequency, 70℃ of extraction temperature, 95% of ethanol volume fraction, and 60 min of extraction time. The optimal temperature of supercritical CO_2 extraction of nicotine with pyrolysis was 450℃, and the optimal concentration of entraining agent was 70%. The extraction temperature was recommended to be 325℃ with the extraction pressure of 30 MPa, the extraction time of 2 h. Compared with the traditional method, the Pyrolysis-supercritical CO_2 co-extraction method increased the extraction rate to over 90%. These methods provided effective means and technical support for the comprehensive use of waste tobacco resources.

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