Study on the Composition and Super Critical Carbon Dioxide Extraction of Chinese Hickory (Carya cathayensis Sarg.) Kernel Oil

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Abstract

Chinese hickory (Carya cathayensis Sarg.) is one of the most productive woody oil-bearing plant in China. Four different extraction methods were explored and supercritical CO$_2$ was selected as green and non-toxic solvent to extract Chinese kernel oil. Four experiment factors, particles size, extraction time, extraction temperature and extraction pressure, were selected to carry out the single factor experiments. According to the results of orthogonal experiments design, the condition of B$_3$C$_2$A$_2$D$_2$ was the optimum reaction parameters. When the experiments were carried out at the optimum parameters, the yield of Chinese hickory kernel oil was 74.5%. The oil fatty acids profiles were analyzed, the results showed that total unsaturated fatty acids were 93.05%. Among them, oleic acid was 66.5 ± 0.44 as the main component. Saturated fatty acids were 6.92 ± 0.21.

Keywords

Chinese Hickory, Extraction, Supercritical CO$_2$, Fatty Acids

1. Introduction

In recent years, food safety draws a lot of attention. In ordinary daily life, edible oil is usually used to cook almost in every dish. However, some unhealthy cooking styles like fried and high temperature boiling will lead to some hazard chemicals produced, and these substances such as dioxine, polycyclic aromatic hydrocarbons which could raise high risk of cancer. Therefore, to prevent toxic substances forming in manufacturing edible oils is essential in processing period. The plant-based oils that obtained from fruits seeds and vegetables are usually processing by screw pressing, solvent extraction and cold pressing [1] [2] [3].
Screw pressing is a very efficient method with lower contents of oil in cake of residues of raw materials. However, during the whole screw pressing, temperature of screw process is high (100°C - 200°C). And at the high temperature, toxic chemicals will be formed; color of oil will grow darker; flavor of oil will be degraded; nutritional value of oil will be decreased; and taste of oil will grow bad. Cold-pressed applied to process the oils through a combination of grinding and low heat is healthier than conventionally method made oil with screwing press. Solvent extraction is high efficient and needs ambient temperature. However, solvents are difficult to remove all out of the oil. In conventional extraction, raw materials are heated to very high temperatures, sometimes up to 230°C, which changes their chemical structure.

In recent years, many researchers pay more attention to supercritical fluid extraction technology as an important alternative to the traditional separation methods. Supercritical fluid extraction technology has been growing rapidly in many fields [4] [5] [6], since the before mentioned limitation can be avoided [7]. Compared to traditional method, supercritical fluid extraction technology has advantages: mild critical point (critical temperature 31.7°C and critical pressure around 7 MPa) green, high yields, no residue, non-toxicity, low cost and high selectivity to non-polar molecules [8]. Super critical CO₂, a well-known technology used to extract high added value compounds from many different sources, has been the most used supercritical solvent, because the compounds can be obtained without contamination by toxically organic solvents and without thermal degradation [9].

Chinese hickory (Carya cathayensis Sarg.) as a native species in China is one of the most productive woody oil-bearing plant according to the “Compendium of Materia Medica” [10]. Chinese hickory has been used to keep fit and prevent cardiovascular diseases and treat cough, stomach ache, and cancer in Asia and Europe [11]. Chinese hickory kernels contain 60% to 70% oil and the oil contains approximately 7% saturated, 20% monounsaturated and 73% polyunsaturated fatty acid; Besides oleic acid, other compositions include a moderate amount of linoleic acid 20% and low concentrations of palmitic 4%, stearic 2%, and linolenic acid 5% [12] [13] [14].

In this study, firstly supercritical CO₂ as the solvent was applied to extraction kernel oil of China hickory and several extraction parameters such as pressure, temperature and particle size were studied on the extraction yield of kernel oil of Chinese Hickory. Secondly, orthogonal experimental method was adopted to optimize the yields of extraction. Last, the fatty acid profiles of Chinese hickory samples from extraction by supercritical CO₂ were determined by GC-MS.

2. Experimental

2.1. Raw Materials and Chemicals

Kernel of Chinese hickory was purchased from Lin’an Hangzhou City, China and stored in refrigerator at 2°C - 4°C till treatment. The kernels were dried at 105°C for 24 h, to a moisture content of 7% - 8% then milled and screened using
a milling machine (Hebi Metallurgy Machinery Equipment Co., Ltd. Hebi, China) to obtain powder with a diameter under 0.3 mm, before the experiments. The chemicals and reagents were bought all from aladdin chemical company China.

2.2. Solvent Extraction

Oil was extracted from 5 g of Chinese hickory (dried and milled) using n-hexane. The extractions were carried out in a Soxhlet apparatus with 50 mL of boiling n-hexane for 8 h. The mixture of oil and hexane was collected, concentrated under vacuum, and dried for 5 min at 105˚C [15].

2.3. Moisture Content

Moisture content of ground Chinese hickory kernel was determined by air oven standard methods recommended by the AOAC. Briefly 5 g of sample in triplicate was dried in hot air oven at 130˚C - 133˚C for 2 h. After drying, the dried sample was again weighed. The following Equation (1) was used for calculating the moisture content [16].

\[
\text{Moisture content (\%)} = \frac{\text{Initial weight of sample} - \text{Weight sample after heat}}{\text{Initial weight of sample}} \times 100. \tag{1}
\]

2.4. Determination of Yield of Chinese Kernel Oil

Oil yield was determined per kernel sample (100 g) on a dry weight basis, using the following Equation (2):

\[
\text{Oil yields (\%)} = \frac{\text{Weight of extracted oil}}{\text{Weight of kernels}} \times 100. \tag{2}
\]

2.5. The Fatty Acid Profiles

The fatty acid composition of Chinese hickory kernel oil was determined by first converting the oil into fatty acid methyl esters [17]. Briefly, mixture of 1 ml of n-hexane and 200 l of 2 M sodium methoxide were added into 40 mg of Chinese hickory kernel oil, then mixed with 200 ml of 2 N HCl in water bath at 50˚C for a while.

GC-MS analysis were performed on a GC-MS HP7890B gas chromatograph equipped with a HP-5973 mass selective detector using a polar capillary column (HP-Innowax polyethylene glycol, 30 m × 0.25 mm id., 0.25 m film thickness HP-wax capillary column (5% Phenyl Methyl Siloxane, Agilent 19091 J-413) with helium as carrier gas. The detector temperature was 240˚C; Column temperature program was 120 for 2 min and then programmed to 180˚C for 2 min at 10˚C/min, then increased to 280˚C for 10 min at 3˚C/min. Fatty acids compounds were identified by comparing their mass spectra with the mass spectra from MS database (NIST 05, WILEY 7).

2.6. Supercritical Fluid Extraction Equipment and Procedure

The supercritical CO₂ extraction experiments were carried out in a lab-scale ex-
traction systems (Applied Separations Inc. PA, USA), whose diagram is shown in Figure 1. The carbon dioxide used was CO₂ (purity 99.99%) and was supplied by Hangzhou gas Co., Ltd. China. The maximum specifications of this experimental setup are 150°C and 50 MPa. The extractor has a volume of 26.5 mL with 1/2” internal diameter. In a typical experiment, around 20 g of Chinese hockery were placed in the extractor, which was pressurized with CO₂ up to the extraction pressure. Then, the solvent flowed at the desired pressure and temperature (at a mass rate of 0.14 ± 0.02 kg/h) for the desired time (maximum 3 h). Different combinations of pressure and temperature were tried in order to study their influence on the extraction performance [18].

2.7. Orthogonal Experiments Designing and Data Analysis

After the single factor experiments, the effect of parameters on supercritical CO₂ extraction was simultaneously studied using a four factor design, with three levels for each factor (see Table 1). The selected factors were particles size of Chinese hickory, extraction temperature, extraction pressure, extraction time and extraction temperature. The orthogonal table L₉(3)⁴ was designed and three levels were set for each factor, and the boundary values for the levels were determined in pilot experiments [19].

Each extraction experiments was analyzed there separate times. Averages of the data and their standard deviations were calculated and presented as error bars. An ANOVA analysis was performed to evaluate statistically the influence of cold pressing and CO₂ extraction at different parameters. Values are presented as means ± standard deviations.

3. Results and Discussion

3.1. Extraction of Chinese Kickory Oil

Oil extraction method was divided into 2 kinds. One kind is physical method e.g. cold press and screw press. Cold press had drawn a lot of attention due to its application with low temperature and ecofriendly. In comparison with cold process, screw process which leads to high temperature were very effectively and less residue oil in cake. However, screw process is prone to produce hazard chemicals. The other kind is solvent method like acetone, hexane, No. 6 solvents etc. Solvent extraction with soxhlet apparatus could obtain the theoretical yield of plant's fruit, seed and so on. Nevertheless, solvents in oil are extremely removed out thoroughly. As shown in Figure 2, in this paper, four extraction methods applied to extract Chinese hickory oil were accessed according to their oil yields. Among them, hexane extraction was showed high yield (75% ± 4.3%) of Chinese kernel oil. Yield (52% ± 2.5%) of Chinese oil obtained from cold express method was lower than other methods. Supercritical CO₂ extraction was a green, no residues and keeping compositions of Chinese hickory kernel oil fresh. Yield of Chinese hickory kernel oil obtained by Supercritical CO₂ extraction could reach 66% ± 3.6% at the comparison experiments.
3.2. The Effects of Particles Size

In this paper, super critical CO₂ extraction were evaluated by studying reaction factors such as particle size of Chinese hickory kernel, reaction time, reaction pressure and reaction temperature. The moisture in kernel of Chinese hickory was reduced at dry cabinet at 105 °C for 8 h to remove excess moisture. The crashed kernels of Chinese hickory were good for solvents to penetrate into inner kernels of Chinese hickory and curtail diffusion distance between solvent molecules and solid particles. And small particles size were as well as benefit for
extraction process, theoretically, extraction speed will get more faster, when particles size are smaller. However, when particles of raw materials were too small, the raw materials were prone to be pressed very tightly; and the gas tube of experimental equipment was apt to be blocked. As shown in Figure 3, particles size at 20 to 40 mesh were optimum for experiments.

3.3. Effects of Extraction Time

In timeline, supercritical extraction has 3 phases: begin phase, transfer phase and end phase. At the begin phase, particles contacted insufficiently with solvents, in this situation, the solvent was sufficient to particles. So, at begin phase, the extraction speed was very fast as shown in Figure 4. Followed, at transfer phase, more solvents contacted to particles of Chinese hickory kernel oil, therefore, more oil were obtained. The yields of Chinese hickory oil grew rapidly. At the end of phase, supercritical CO₂ extraction grew slowly till no oil extracted from Chinese hickory. The optimum time was 2.5 h, 3 h and 3.5 h.

3.4. Effects of Extraction Pressure

During the process of supercritical CO₂ extraction, the extraction pressure was the very essential parameter. The solubility of supercritical liquid CO₂ was improved as the pressure of liquid CO₂ increasing. The reason was that as the press of critical CO₂ increasing, not only the solubility of CO₂ improved, but also the distance between particles of Chinese hickory kernel and solvent molecules were cut down. In this circumstance, efficient of extraction were increased and in favor of Chinese oil extraction. Furthermore, increasing reaction pressure also could decrease the reaction time and made the process safer and swift. As shown in Figure 5, at different extraction temperature of Chinese kernel oil were performed. When extraction pressure was less than 30 Mpa, the yields of Chinese hickory kernel oil increased, as the extraction pressure increasing. When extraction pressures were more than 30 Mpa, the yields of the kernel oil increased very slowly.

![Figure 5](image-url)
Figure 4. Effects of extraction time. Data are presented as means, the error bars indicate standard deviations (n = 3).

Figure 5. Effects of extraction pressure. Data are presented as means, the error bars indicate standard deviations (n = 3).

3.5. Effects of Extraction Temperature

Extraction temperature was an important factor for supercritical CO2 extraction. As reaction temperature raising, the heat refluxing becomes fierce, meanwhile the collide possibility between molecules of solvent and molecules in starting materials increased. Therefore, the solubility of liquid CO2 improved. However, as reaction temperature soaring, the density of liquid CO2 became lower, and the liquid CO2 carried less oil from Chinese hickory kernel oil. As shown in Figure 6, in the experiments, below 40˚C, the yields of Chinese hickory kernel oil increased as the extraction temperature rising. However, above 40˚C, the yields of Chinese hickory kernel oil slightly decreased as the extraction temperature rising. Thus, 25˚C to 35˚C were optimum extraction temperature.

3.6. Orthogonal Experiments Design

Based on the single parameters experiment, four relevant factors were investigated: particles of Chinese hickory kernel, extraction time, extraction pressure, extraction temperature. The orthogonal table L9(3)4 was designed as shown in Table 1. Three levels were set for each factor. According to pilot experiments, 35˚C, 40˚C and 45˚C were selected as three levels for orthogonal experiments. And the extraction time selected 1.5 h, 2 h and 2.5 h. From the pilot experiments
Figure 6. Effects of extraction temperature. Data is presented as means, the error bars indicate standard deviations \((n = 3)\).

Table 2. Results of orthogonal experiments.

| Trial No. | A   | B   | C   | D   | Yields (100%) |
|-----------|-----|-----|-----|-----|---------------|
| 1         | 1   | 1   | 1   | 1   | 38.2          |
| 2         | 1   | 2   | 2   | 2   | 60.4          |
| 3         | 1   | 3   | 3   | 3   | 65.3          |
| 4         | 2   | 1   | 2   | 3   | 63.2          |
| 5         | 2   | 2   | 3   | 1   | 67.5          |
| 6         | 2   | 3   | 1   | 2   | 67.8          |
| 7         | 3   | 1   | 3   | 2   | 62.2          |
| 8         | 3   | 2   | 1   | 3   | 61.8          |
| 9         | 3   | 3   | 2   | 1   | 71.2          |
| K1        | 230.6 | 189.3 | 215.2 | 233.6 |               |
| K2        | 257.8 | 253.3 | 261.3 | 248.7 |               |
| K3        | 210.3 | 271.2 | 242.1 | 234.7 |               |
| R         | 33.21 | 76.1  | 43.2  | 21.2  |               |

K1: the effect of each factor at level \(i (i = 1, 2, 3)\); R: range.

Table 3. Fatty acid profiles and contents of Chinese hickory kernel oil*.

| Fatty acids                  | Contents** (100%) |
|------------------------------|-------------------|
| Palmitic acid                | 4.7 ± 0.07        |
| Palmitoleic acid             | 0.13 ± 0.04       |
| Stearic acid                 | 2.17 ± 0.16       |
| Oleic acid                   | 66.5 ± 0.44       |
| Linoleic acid                | 24.1 ± 0.35       |
| Linolenic acid               | 2.3 ± 0.21        |
| Arachidic acid               | 0.12 ± 0.02       |
| \(\text{Cis-11}-\text{eicosenoic acid}\) | 0.24 ± 0.12 |
| Total saturated fatty acids  | 6.92 ± 0.21       |
| Total unsaturated fatty acids| 93.05 ± 0.12      |

*Each sample were performed 3 duplicates, and the value were demonstrated as mean ± standard error;
**The contents of the fatty acid based on the whole fatty acid in the Chinese hickory kernel.
(see Figure 5) the extraction pressure was very important parameters for extraction, and the suitable pressure were 25 Mpa, 30 Mpa and 35 Mpa. According to the results of orthogonal experiments design (see Table 2), during the 4 factors of the experiments, Parameters of extraction time had the biggest range (R = 76.1) and extraction pressure, particles size and extraction temperature were as followed. These results showed that extraction time and extraction pressure had larger effects than extraction temperature and particle size. According to the tale of results orthogonal experiments design, the condition of B₃C₂A₂D₂ was the optimum reaction parameters. At the optimum parameters, the yield of Chinese hickory kernel oil was 74.5%.

3.7. Fatty Acid Profiles

Fatty acid profiles extracted from the optimum conditions, then the fatty acids were methylation to investigation on GC-MS, The result was shown in Table 3. Eight fatty acids were identified in Chinese hickory kernel oil. Unsaturated acids were predominant (total unsaturated fatty acids were 93.05%). Among of them, Oleic acid was 66.5 ± 0.44 as the main components. Saturated fatty acids were 6.92 ± 0.21. This kind of fatty acid profiles is very similar with olive oil. Besides saturated and unsaturated oil, there are still have Linolenic acid, Arachidic acid and Cis-11-eicosenoic acid, and these fatty acids also very good for human health [20].

4. Conclusion

In this research, the different extraction methods were explored and supercritical CO₂ selected as green and non-toxic solvent to extract Chinese kernel oil. Four experiment factors were selected to carry out the single factor reaction. Through single factor reaction, four levels are selected respectively. According to the results orthogonal experiments design, the condition of B₃C₂A₂D₂ was the optimum reaction parameters. When the experiments were carried out at the optimum parameters, the yield of Chinese hickory kernel oil was 74.5%. The oil fatty acids profile were analyzed, the results showed that total unsaturated fatty acids were 93.05%. Among them, oleic acid was 66.5 ± 0.44 as the main component. Saturated fatty acids were 6.92 ± 0.21.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.
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