Optimization of essential oil yield from Vietnamese green pepper (Piper nigrum) using hydro-distillation method

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Abstract. In this study, the essential oil of green pepper collected from Vung Tau City, Vietnam was recovered by hydro-distillation technique, giving the yield of approximately 0.75% yield on optimal conditions. Single factor investigation showed that optimal conditions of the extraction process consisted of water and material ratio of 5:1 mL/g, extraction time of 180 min, flow rate of 2.5 mL/min, size of material of puree, time for soaking of 3 hours. Nineteen components were identified in the obtained Piper nigrum essential oils using GC-MS. The main components of essential oils were Caryophyllene, 3-Carene, β-Pinene, α-Pinene, Sabinene, and Elemene.

1. Introduction

Nowadays, herbs and spices have been receiving a great deal of public attention due to biological activities and various functions. Essential oils (EOs) from plants play a crucial role in different fields...
such as food, cosmetics, pharmaceuticals and so on [1-7]. Essential oils contain aromatic polypropanoid compounds and terpenes, which obtained from the shikimic acid and acetate- mevalonic acid pathways. *Piper nigrum* L. (Black pepper), belonging to the *Piperaceae* family, which cultivated mainly in tropical and subtropical countries such as Indonesia, Malaysia, Vietnam. Black pepper is commonly used in different fields such as agronomics, pharmaceuticals, food. The previous phytochemical investigations made on the black peppers have proved that they possess a wide variety of compounds like antioxidant, antibacterial, antifungal, anti-inflammatory and anti-tumor properties and insecticidal agents [8-13]. The previous study demonstrates that the main ingredient in the black pepper essential oil was piperine. α- and β-pinene plays an essential role in relieving digestion issues, flavor and aroma. Recent years, the extraction process of black pepper oil has increased attention in the literature due to the wealth of beneficial components. There are different techniques to extract the oil from black pepper including solvent extraction, hydro-distillation, steam distillation and so on. Hydro-distillation is the primary method for obtaining essential oils [14-17]. The hydro-distillation is available in larger scale production due to economic reasons. There are two ways to operate hydro-distillation including passing steam through the sample materials or heating the mixture consisting of material.

The objective of the present research was to investigate the optimal conditions of the hydro-distillation method (material size, the concentration of sodium chloride, the concentration of soak time, time extraction, ratio material to water, air flow rate) and chemical composition of essential oils collected from seeds of black pepper (*Piper nigrum* L.) fruits grown in Vietnam. This paper has the goal of extracting essential oils of pepper widely used in Vietnamese cuisine. After extraction, chemical constituents extracts were characterized by GC-MS.

2. Materials and methods

2.1. Materials and chemical

The materials used during the study were fresh (green) pepper (*Piper nigrum* L.) seeds, which were grown, harvested and processed in Vung Tau Province (10°20’37”N 107°05’43”E), Vietnam. Pepper seeds were ground carefully and gently with a grinder for 1 min. The sample was weighted precisely for each experiment and was used immediately to limit the denaturation of essential oil.

2.2. Hydro-distillation

To extraction the essential oil by hydro-distillation, First, 50g of green pepper seed were washed and ground. Second, the 50g seed is mixed with 1L distilled water. Then, Clevenger extraction was operated for 30-240 min. Next, the raw essential oil was separated to obtain the extraction oil which was dried with Na$_2$SO$_4$. The yield of black pepper oil obtained (%) is calculated by the following formula:

\[
Y(\%) = \frac{\text{Volume of essential oil (ml)}}{\text{Amount of raw materials (g)}} \times 100\% \quad (1)
\]

2.3. Chemical composition analysis GC-MS

In order to determine the chemical composition in the essential oil sample, 25 µL of sample from the optimized process was mixed with 1.0 mL n-hexane and dehydrated with Na$_2$SO$_4$. The instrument was GC Agilent 6890 N, HP5-MS column, MS 5973 inert, head column pressure of 9.3 psi. The gas chromatography-mass spectroscopy (GC-MS) was employed to analyze the chemical composition of the essential oil samples. GC-456 SQ with SCION performance RESTEK Rxi-5ms (30 m x 0.25 mm,
0.25 μm df, bring the gas Helium constant flow rate: 1 mL/min. Injector temperature is 250° C the rate of Division: 30.

3. Results and discussion

3.1. The effect of material size on the yield essential oil

As the outer peels of the green pepper were thick and hard, the efficiency of the extraction process could be hindered without the peeling process. Therefore, it is necessary to perform treatment accordingly to different sizes. Based on Figure 1, the amount of extracted essential oil increased as the size of the pepper decreased. The lowest yield at 0.05% is achieved without peeling, and significantly higher yields, at 0.15% and 0.4% were obtained at size mince and puree, respectively. This result is explained by the fact that when the green pepper is ground, the cells containing essential oil were broken, facilitating the water penetration into the essential oil containing bags.

![Figure 1](image)

**Figure 1.** The influence of material size on the yield of essential oil

3.2. The effect of the concentration of NaCl on the yield of essential oil

The results from the investigation on the concentration of NaCl solution used to immerse the raw material were analyzed in Figure 2. In the extraction process, the mixture of essential oil and water formed the emulsion. When adding salt to the extraction mixture, not only could the loss of essential oil in the form of emulsion be avoided, but the solubility of some non-polar components of essential oil into the water medium could also be reduced. Besides, the NaCl salt also plays a vital role as an electrolyte that increases the density and polarization of water, which makes the separation of essential oil from water easier. As NaCl concentration reaches 2%, oil yield was found to be declining. This result could be explained by the osmotic pressure difference exerted by the higher solute concentration in the external environment in comparison with that inside the oil-containing cells. This effectively causes the water inside the cell to osmose out, hindering the separation of essential oils from the material. Figure 3 displayed that in the same extraction conditions, the sample with a NaCl concentration of 2% gave the best results with the yield of 0.4%, and at a concentration of 5% achieved at approximately 0.3%. Therefore, the optimal concentration of NaCl is selected as 2% for the best yield of the essential oil.
Figure 2. The influence of the concentration of NaCl solution on the yield of essential oil

3.3. The effect of the concentration of soak time on the yield of essential oil

From Figure 3, the soak time of the NaCl 2% solution before extraction enhances the extract efficiency due to the time needed to induce the penetration of essential oil into the environment. On the other hand, prolonged exposure to salt also assists in breaking down oil bags, and high water emulsion could facilitate the transport of oil by steam. The results showed that oil yield was increasing with the soak period, reaching the maximum yield at 3h. Afterward, increasing soak time reduces extraction efficiency. Therefore, 3 hours of soak time was selected for the highest essential oil of 0.4%.

Figure 3. The influence of the concentration of soak time on the yield of essential oil
3.4. The effect of extraction time on the essential oil yield

Figure 4 shows the time influence on the essential oil yield from 30 to 300 minute. The highest essential oil content was obtained at 180 minutes, achieved 0.75%. When extending the extraction time to a certain limit, the amount of recovered oil does not increase further and may affect the quality of the product because some substances can be denatured due to prolonged exposure with temperature. It is also crucial to determine time since it concerns energy consumption and production cost [18]. Therefore, 180 min was chosen as the extraction time for the next survey.

![Figure 4. The influence of extraction time on the yield of essential oil](image)

3.5. The effect of air flow rate on the yield of essential oil

Figure 5 illustrates the influence of air flow rate on the yield of essential oil. For air flow rate, with an increase from 2.1 mL/min to 3.7 mL/min, the highest extraction essential oil yield increased from 0.7% to 0.75%, respectively. However, the amount of essential oil nearly constant at 0.125% when rising air flow rate at 2.5 mL/min and 3.7 mL/min. To better understand this factor, this air flow rate, which is a parameter that shows the amount of heat impacted on the flask containing the material causing the water in the tank to rise in temperature until evaporation, then through the condenser and stored on the Clevenger. Moreover, increasing the amount of heat will cause the essential oil to drain more and improve the efficiency of the essential oil. To a certain extent, the escape rate for essential oils in saturated essential oil bag cannot be increased [19].
3.6. The effect of the material and water ratio on the essential oil yield

In the steam extraction, when heating the mixture of water and material, the water vapor permeates the epidermis, which contains essential oils, breaks down the essential oils, and attracts the oil by steam. If the amount of water is insufficient to dissolve the colloids and salt wrapping the pouch of the essential oil, the oil is unable to escape. As can be seen from Figure 6, more water for extraction will cause higher diffusion of oil into the water, leading to enhanced solubility and improved the yield of soluble components.

On the contrary, excess water could dissolve or emulsify the essential oil, reducing the amount of essential oil yield and the economic efficiency of the distillation die to increased energy consumption and extraction duration. Based on Figure 6, raising material to water ratio 1:2 to 1:5 (g/mL) will increase the extraction of oil yield to approximately 0.75%. The higher solution of the solution up to 1:10 (g/mL) causes essential oil yield to decrease sharply. Therefore, the ratio of 1:5 (g/mL) was chosen, it could save significant water used and in turn brings high economic value [20].

**Figure 5.** The influence of air flow rater extraction on the yield of essential oil
3.7. The result of GC-MS

The compositional data of the pepper oils derived by GC-MS analyses, extracted at the optimized conditions are assembled in Table 1. It was found that 19 compounds in pepper oils were identified by GC-MS analysis. The most dominant were: 3-carene (35.21%), D-limonene (21.54%), β-Caryophyllene (10.05%), β-pinene (9.17%), Sabinene (7.37%), α-pinene (4.45%) and Elemene (4.09%).

For pinene, the compound was found in lower quantity in comparison with black pepper from India when extracted with the same conditions. It is also worth noting that unlike other green pepper cultivars, Vietnamese green pepper oil has a very low component α-phellandrene (0.07%), while in India this component has a content of up to 14.9 %, it is considered the main ingredient in essential oil in here.
Table 1. Composition of essential oil from peppers seed

| Compounds            | Formula | This Study | India HD |
|----------------------|---------|------------|----------|
| α-pinene             | C₁₀H₁₆  | 4.54       | 8.5      |
| β-pinene             | C₁₀H₁₆  | 9.17       | 10.4     |
| Sabinene             | C₁₀H₁₆  | 7.37       | -        |
| α-phellandrene       | C₁₀H₁₆  | 0.07       | 14.9     |
| 3-carene             | C₁₀H₁₆  | 35.21      | -        |
| α-cymene             | C₁₀H₁₆  | 0.86       | -        |
| β-myrcene            | C₁₀H₁₆  | 2.84       | 6.1      |
| D-limonene           | C₁₀H₁₆  | 21.54      | 11.9     |
| Terpinolene          | C₁₀H₁₆  | 0.03       | 0.50     |
| Linalool             | C₁₀H₁₆O | 0.32       | -        |
| α-terpineol          | C₁₀H₁₆O | 0.11       | 0.5      |
| α-cubebene           | C₁₅H₂₄  | 0.08       | 0.9      |
| Copaene              | C₁₅H₂₄  | 0.96       | -        |
| β-Caryophyllene      | C₁₅H₂₄  | 10.05      | 9.1      |
| Humulenene           | C₁₅H₂₄  | 2.10       | 0.1      |
| Germacrene-D         | C₁₅H₂₄  | 0.15       | -        |
| α-selinene           | C₁₅H₂₄  | 0.50       | 1.5      |
| β-bisabolene         | C₁₅H₂₄  | 0.01       | 1.9      |
| Elemene              | C₁₅H₂₄  | 4.09       | 0.9      |

4. Conclusion

In this study, the conditions for optimal hydro-distillation extraction of essential oil from Vietnamese green pepper seeds were investigated. Moreover, GC-MS analyses also were used to reveal the chemical composition of the produced essential oil. Optimal conditions included an extraction time of 180 min, the air flow rate 2.5 mL/min and the material-to-water ratio 1:5 g/mL. Moreover, 19 compounds of essential oil in green pepper have been identified using GC-MS. The results showed that 3-carene
(35.21%), D-limonene (21.54 %), β-Caryophyllene (10.05 %), β-pinene (9.17 %), Sabine (7.37 %), α-pinene (4.45 %) and Elemene (4.09%) are the major constituents of the essential oil.

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