Extraction of Clove Essential Oil by Microcapsule Aqueous Two-phase System

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Abstract: Traditional method to prepare clove oil is by steam distillation of crude clove oil. However, the major drawback of this method is that the densities of clove and water are very close to each other and further extraction by organic solvents is inevitable. To solve this problem and follow the requirement of environmental benign operation, a microcapsule β-cyclodextrin in aqueous two-phase system is developed to extraction of crude clove essential oil with higher selectivity and purity than steam distillation. Moreover, this stable clove capsules with β-cyclodextrin may have broad applications in food, medication, cosmetic and tobacco industries.

Key words: Clove, essential oil, aqueous two-phase extraction, microcapsule, β-cyclodextrin.

Introduction

Clove is a kind of evergreen tree that belongs to the rosewood cherries. Due to the special and strong aroma, clove oil has been widely used in food additives, tobacco, flavorings and other fields. In general, clove oil is prepared by steam distillation, however, one major drawback of conventional steam distillation method is that the density of clove oil is very close to water, further extraction by organic solvents, for example, petroleum ether is necessary. No doubt, the use of organic solvents may cause environmental pollutions. At present, as people pay more and more attention to environmental issues, developing a new method for in place of that avoiding the use of organic solvents to extract clove oil is urgent and of highly importance.

The aqueous two-phase extraction systems are green and clean alternatives for conventional organic-water solvent extraction systems. Both the two phases are composed of water and non-volatile components, such as polymers and inorganic salts, therefore, aqueous two-phase extraction is more suitable for separation of biomaterials which may degrade in the presence of organic solvents. On the other hand, microcapsule technology is frequently used to enclose solids, liquids, or gases inside a micrometric wall. Microencapsulation can be used to increase the stability and life of the essential oils being encapsulated, reducing volatility and controlling the rate of release and so on. Technically, the combination of the two technologies to extract plant oil is practical and feasible, however, related researches remain largely unexplored. Therefore, in this paper, we wish to report a new approach that combines these two technologies, for the separation of clove essential oil from the clove extract by β-cyclodextrin in aqueous two-phase system. In our method, we used sodium sulfate and cyclodextrin to form an

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aqueous two-phase system, which gave good separation of clove essential oils because it was found that the major component was wrapped in β-cyclodextrin aqueous phase. Upon a simple separation, the β-cyclodextrin inclusion complex with essential oils was obtained, dried in vacuo, and then treated with ethanol, finally, the ethanol solvent was evaporated and clove essential oils were obtained with high purity.

Materials and methods

Materials and reagents

Crude Clove oil (Anyang ManTianxue Food Co. Ltd., Anyang, China); β-cyclodextrin (Beijing Xinghuo Chemical Factory, Beijing, China); eugenol (AR), trans-caryophyllene (AR), eugenol acetate (AR), ethanol (95 %, Reagent grade), dichloromethane (Reagent grade), anhydrous sodium sulfate (Food grade) (Sinopharm Chemical Reagent Co., Ltd, Beijing, China).

Main instruments and equipment

HP6890A/5973N GC/MS (Agilent Technologies); JEOL JSM-6510LV scanning electron microscope (SEM, Tokyo, Japan); Rotary Evaporator RE-51AA (Shanghai YaRong biochemical instrument factory); SHZ-D Circulating vacuum pump (GongYi YuHua Instrument Co. Ltd.); Thermostat Water Bath Tank Series HH-6 (Changzhou Aohua Instrument Co. Ltd.); Electric Heating Jacket (GongYi YuHua Instrument Co. Ltd.); Heating magnetic whisk (IKA)

Experiments

Procedures

Separation of clove essential oil by aqueous two-phase system

100 g of crude clove oil was added to 40 % ethanol aqueous solution (500 g), the resulting mixture was treated by ultrasonication for 40 min. Then, the above mixture was added 20 % β-cyclodextrin water solution and 15 % food grade sodium sulfate. The mixture was stirred (1200 r/min, heating magnetic whisk, IKA) in a water bath at 45°C for 6 h and then set down until two layers were formed. The β-cyclodextrin layer was separated, filtered and dried, and clove oil microcapsules were obtained. The clove oil microcapsules were then subjected to 95 % ethanol and stirred at 60°C for 1 h to release the extract. The mixture was filtered and the filtrate was concentrated on a rotary evaporator (0.01 MPa, 40°C, Shanghai YaRong), the clove essential oils were obtained as a clear liquid and were tested by GC-MS and 1H NMR without further purification.

Analytical sample preparation

Four flasks were charged with water (40 mL) and ethanol (10 mL), respectively. 0.5 g of crude clove oil, eugenol, eugenol acetate and trans-caryophyllene were added to each of the above flask. Then 10 g β-cyclodextrin and 7.5 g food grade sodium sulfate, were added to each of the above flask. The flasks were all stirred (1200 r/min, heating magnetic whisk, IKA) in a water bath at 65°C for 30 min, then the β-cyclodextrin layer was separated, put in refrigerator at 4°C overnight, white precipitate was formed and the mixture was filtered (Whatman® qualitative filter paper, 30-50 μM) and white powders were obtained after drying in air. The solids were further dried by high vacuum pump (40°C, 24 h, SHZ-D Circulating vacuum pump, GongYi YuHua) and put in a well-sealed zip-lock bag.

Analysis of volatile and semi-volatile constituents of essential oils

GC / MS conditions: Column: RB-InoWax (30 m × 0.32 mm × 0.25 μm) capillary column; Injection temperature: 250°C; Splitless injection; Carrier gas: He, 1 mL/min; Temperature rise program: 50°C (2 min) to 10°C / min rose to 230°C (maintaining for 0 min); Transmission line temperature: 400°C; Ion source: EI source; electron energy: 70 eV; Scanning range: 50-650 amu; using NIST08 library to determine the aroma components, and the area of the corresponding peaks are calculated to determine the relative percentage of each components in the clove oil.

Microcapsule Infrared Spectroscopy Analysis

100 mg KBr and 1 mg β-cyclodextrin inclusion complexes were thoroughly grounded into fine powders, mixed and homogenized by grinding, and then pressed into a 1 mm thick sheet and scanned. The scanning wave number ranges from 400 to 4000 cm⁻¹.
SEM analysis of clove capsules

The morphologies of β-cyclodextrins and their inclusion complexes were observed with a JEOL JSM-6510LV scanning electron microscope (SEM, Tokyo, Japan). They were mounted on a freshly cleaved glass slide using carbon adhesive tape and sputter-coated with a mixture of gold and palladium (60:40) in an argon atmosphere under low pressure.

Results and discussions

Analysis of volatile and semi-volatile constituents of essential oils

According to the established analysis methods described, the volatile aroma components of crude clove oil and the microcapsule aqueous two-phase were analyzed. Clove essential oil was analyzed by capillary gas chromatography. The relative percentage of volatile and semi-volatile components of the chemical components in clove was calculated by $^1$H NMR and the results were shown in Scheme 1 (see SI for detailed information).

As can be seen from Scheme 1, Scheme 2 and Table 1, the major components of crude clove oil and extracts derived from microcapsule aqueous two-phase vary greatly. There are three components in the clove oil using steam distillation, including eugenol (70.42 %), trans-caryophyllene (14.08 %) and eugenol acetate (15.50 %). In contrast, the microcapsule aqueous two-phase method gave the product containing only one major component, eugenol (90.77 %), together with very small amount of eugenol acetate (9.23 %). We envisage that cyclodextrin can selective form encapsulations with eugenol and eugenol acetate ingredients in the extraction process, making an excellent separation of eugenol from clove oil.

Infrared Spectroscopy of Clove Microcapsules

Cyclodextrin, inclusion complex of cyclodextrin with clove oil, and inclusion complexes of cyclodextrin with eugenol, trans-caryophyllene and eugenol acetate were tested by IR.

As shown in Scheme 3, the infrared spectra of the inclusion complexes were similar, indicating that the skeletal structure of β-cyclodextrin did not change. However, new peaks were observed in IR spectroscopy of inclusion complexes of β-cyclodextrin with eugenol and eugenol acetate. The new peak appeared at 1515 cm$^{-1}$, which was the characteristic peak of eugenol. In addition, the new peaks of clove naproxate and β-cyclodextrin inclusion complex appeared at 1767 cm$^{-1}$ and 1510 cm$^{-1}$, which were characteristic peaks of eugenol acetate. There was no new characteristic peak in the β-cyclodextrin inclusion complex of caryophyllene, which indicated that caryophyllene can’t form stable inclusion complex with β-cyclodextrin. Though eugenol and clove acetic acid phenol ester could bind with β-cyclodextrin in the same content, based on the above results, the interaction between eugenol and β-cyclodextrin is much stronger, giving a highly selective extraction of eugenol from clove oil.

SEM analysis of clove oil microcapsule

Cyclodextrins and their inclusion complexes were tested by scanning electron microscopy, and the results are listed in Scheme 4.

It can be seen from the figures that the surface of β-cyclodextrin inclusion complex with eugenol is smoother and appears to be a tight, regular cube state, indicating that a more stable clathrate is obtained. The surface of β-cyclodextrin inclusion complex with trans-caryophyllene is uneven, and shows a loose, irregular state, suggesting no stable phase is formed. In addition, β-cyclodextrin inclusion complex with eugenol acetate is partially regular, and the formed phase is not as stable as eugenol. While the β-cyclodextrin inclusion com-

### Table 1. Major components of clove oil

| Entry | Retention time (min) | Component           | Relative abundance (%) |
|-------|----------------------|---------------------|------------------------|
|       |                      |                     | Crude clove oil | Microcapsule aqueous two-phase system |
| 1     | 13.59                | Eugenol             | 70.42                | 90.77                |
| 2     | 14.66                | trans-Caryophyllene | 14.08                | -                    |
| 3     | 15.62                | Eugenol acetate     | 15.50                | 09.23                |
Scheme 1. GC of crude clove oil

Scheme 2. GC of clove oil obtained by microcapsule aqueous two-phase system
Scheme 3. IR of inclusion complexes

Scheme 4. SEM analysis of β-cyclodextrin inclusion complex
(A: clove oil; B: eugenol; C: trans-caryophyllene; D: eugenol acetate)
plex with clove oil shows a partially regular state. The results of electron microscopy showed that eugenol combined the best with β-cyclodextrin, which was consistent with the results of GC-MS and 1H NMR analysis.

Conclusion

In summary, we have developed a novel aqueous two-phase system for facile separation of eugenol and eugenol acetate from crude clove oil, including three main components, eugenol (70.42 %), trans-caryophyllene (14.08 %) and eugenol acetate (15.50 %). According to the IR analysis, β-cyclodextrin can selectively extract eugenol and eugenol acetate. In addition, the SEM analysis shows that the interaction between eugenol and β-cyclodextrin is stronger than eugenol acetate, and the interaction between trans-caryrill and β-cyclodextrin is very weak. Because the content of eugenol acetate in the clove oil is much lower than that of eugenol, so that our microcapsule aqueous two phase process give a highly selective extraction of eugenol (up to 90.77 %). The extraction and separation process is carried out under low temperature and in water to avoid the usage of organic solvents. We believe that our method is a green and practically effective essential oil extraction method.

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