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Extraction of squalene from camellia oil by silver ion complexation

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ABSTRACT

Separation of squalene, a type of terpenoid hydrocarbons with important biological properties, from camellia oil by silver ion complexation was studied. We showed that the UV–visible absorption wavelength of squalene in $AgNO_3$-methanol solution was obviously red-shifted, and the infra-red vibration wavelength of C=C bond slightly decreased, indicating that complexation between squalene and Ag^+ took place. The distributions of squalene in $AgNO_3$ methanol solution/petroleum ether under different conditions were further investigated. We found that the fraction of squalene in $AgNO_3$-methanol phase was enhanced with increased percentage of methanol and $AgNO_3$ concentration and decreased extraction temperature. The distribution coefficient of squalene was up to 7.87 under optimum extraction conditions with 70% methanol (v/v), 0.6 mol/L $AgNO_3$, reaction time of 12 h, and at the temperature of 0 °C. Furthermore, under the optimum conditions, extraction of squalene from the unsaponifiable portion of camellia oil exhibited high selectivity with a raffinate rate and a back-extraction rate of 73.9% and 72.5%, respectively. The purity of extracted squalene was 37.8%, 108 times more concentrated than that in the starting material.

1. Introduction

Squalene, 2, 6, 10, 15, 19, 23-hexamethyltetracosa-2, 6, 10, 14, 18, 22-hexaene, is a highly unsaturated hydrocarbon and a chain-like triterpene. Squalene has many physiologic functions, such as promotion of SOD activity in vivo, enhancement of the immune responses, sexual vitality, and anti-aging, anti-fatigue, and anti-tumor activities. It is widely used in medicine, foods, cosmetics, and chemical engineering [1–4].

Currently, squalene is primarily obtained from deep-sea shark liver, in which the content of squalene is about 15–80%. However, the source of shark liver is gradually decreased because of long growing period, low reproduction rate, and overfishing of sharks. Therefore, more attention has been paid to the plant resources, such as olive oils and soy bean oils, to obtain squalene.

Nowadays, the general methods for isolating squalene include supercritical fluid extraction [5–8], molecular distillation [9,10], and column chromatography [11–13]. The content of squalene in plant oils is very low, and its composition is complex. Thus, industrial isolation of squalene from plant resources is difficult because the long processing time, small production scale, and high cost.

Silver ion complexation is a separation method based on the complexation reaction between Ag^+ and unsaturated C=C double bonds. The complexation would proceed when the ratio of carbon number of an organic compound to its double bond number, or large π bond number, is less than 6 and there is no steric hindrance [14,15]. This method, with features of high selectivity, mild separation conditions, low reaction energies, simple reactor design, and convenient large-scale production, has been widely used to extract, separate, and concentrate unsaturated fatty acids (esters), such as α-linolenic acid [16], arachidonic acid [17], solanesol [18], DHA and EPA [19,20], triacylglycerols [21].

There are six unsaturated double bonds at C2, C6, C10, C14, C18, and C22 in squalene [11], and substituted methyl groups at C2, C6, C10, C15, C19, and C23. Because those methyl groups are connected with unsaturated bonds, the electron clouds of their C—H bonds would overlap the *p* orbits of double bonds to form hyper conjugation, which further increases the density of electron clouds of the double bonds and drives the complexation reaction. In theory, it is possible to obtain squalene by the method of silver ion complexation. However, the method has not been used to separate squalene in literatures.

Camellia oil is originally obtained from seeds of camellia oleifera abel and used as a cooking vegetable oil in traditional Chinese cuisine. It contains more than 90% unsaturated fatty acid, including 80–83% oleic acid, 7–13% linoleic acid, and abundant squalene of about 12 mg/100 g, equal to that in soy bean oil. Thus, it is another important plant resource to obtain squalene.

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In this work, the distribution property of squalene in the binary extraction system of petroleum ether-$AgNO_3$ methanol solution was investigated. The effects of methanol percentage, extraction temperature, extraction time, and Ag^+ concentration on distribution coefficient were studied and the corresponding complexation reaction process was analyzed. Under optimum extraction parameters, squalene in unsaponifiable matter of camellia oil, which was pretreated by saponification-esterification reactions, was extracted and separated. Our work provides a new route to separate and obtain squalene on a large scale.

2. Experimental

2.1. Reagents

Silver nitrate with purity of ⩾98% was purchased from Sinopharm Chemical Reagent Co., Ltd., Methanol with purity of ⩾98% was purchased from TEDIA High Purity Solvents, USA. Both petroleum ether with purity of ⩾98% and squalene with purity of ⩾99% were purchased from Aladdin Reagent Co., Ltd. Camellia oil was purchased from Yiren Agricultural Development Co., China.

2.2. Instrument

Analytical instruments include Agilent 7890B Gas Chromatograph, Agilent Technologies Inc.; Ultrospec7000 UV–visible Spectrophotometer, GE Healthcare; Thermo Nicolet 360 FT-IR Spectrophotometer, Thermo Nicolet Corporation.

2.3. Procedure

2.3.1. UV analysis of complex

Squalene methanol solution of 10 mL at the concentration of 1.0 g/L was prepared. A certain amount of $AgNO_3$ was added into the solution to the final concentration of 8.0 g/L. The final solution was fully agitated and used for UV–visible spectroscopic analysis. The squalene-methanol solution without $AgNO_3$ and $AgNO_3$-methanol solution were used as control.

2.3.2. FT-IR analysis of complex

Squalene-petroleum ether solution (0.02 mol/L) was mixed with 70% isometric methanol-0.72 mol/L $AgNO_3$ aqueous solution, placed in a cold trap at 0 °C, and allowed to react for 12 h. Squalene complex was analyzed from 600 cm^{-1} to 5000 cm^{-1} after 0 and 12 h by using Thermo Nicolet Fourier transform infrared spectrometer.

2.3.3. Analytic procedure of squalene

The Agilent 7890B GC equipped with a FID detector and a capillary column of HP-5 (30 m × 0.32 mm i.d × 0.25 μm) was used. The detector temperature was 300 °C and the injection temperature was 300 °C. The following temperature program was used for analysis. The initial oven temperature was 130 °C. The temperature was then increased to 230 °C with a ramp rate of 20 °C/min and kept for 2 min. The final oven temperature was 270 °C elevated by a ramp rate of 3 °C. High purity helium (99.99%) with a flow rate of 3 mL/min was used as the carrier gas and high purity nitrogen with a flow rate of 30 mL/min was used as the make-up gas. The sampling amount was 1.0 μL and an external standard method was used to quantitatively measure the concentration of squalene.

2.3.4. Extraction procedure

A solution of squalene-petroleum ether was mixed with an equal volume of $AgNO_3$ methanol. The extraction was carried out at a setting temperature under N_2 atmosphere. The mixture solution would be stratified after reaction at low temperature. The upper stratum of petroleum ether was separated and used to measure the volume and the concentration of squalene.

The distribution coefficient (D) of squalene in the $AgNO_3$-methanol phase and petroleum ether phase can be calculated by the following equation.

$$D = \frac{(C_I \times V_I) - (C_o \times V_o)}{C_o \times V_o}$$

where C_I and C_o are the concentrations of squalene in the petroleum ether phase before and after extraction reaction, respectively; V_I and V_o are the volumes of the petroleum ether phase before and after extraction reaction, respectively.

2.3.5. Pretreatment of camellia oil

Because squalene is unsaponifiable, a saponification-esterification method was used to pretreat camellia oil as following: Camellia oil of 500 mg was put into a 50 mL round flask, in which 0.8 mol/L NaOH-methanol solution of 12 mL was added. The mixture solution was stirred away from the light at the boiling temperature of 30 °C. After agitation reaction for 2 h, saturated NaCl solution of 6 mL and petroleum ether of 10 mL were added into the reaction solution and agitated for another 2 min. Separation of the layers was carried out in a 50 mL separation funnel. Methanol of 4 mL and boron trifluoride-ethyl ether complex of 4 mL were successively added into the separated upper layer. The mixed solution was reacted in agitation at the boiling temperature of 30 °C for 0.5 h. The final solution was centrifuged at 8000 r/min at 4 °C for 5 min to obtain a supernatant.

3. Results and discussion

3.1. UV–visible spectroscopy and FT-IR analysis

The UV–visible spectra of squalene in methanol solution and in $AgNO_3$-methanol solution are shown in Fig. 1. The maximum absorption wavelength of squalene in methanol is at 218 nm, which is close to the maximum absorption wavelength of $AgNO_3$ in methanol (220 nm). After addition of $AgNO_3$ to the squalene methanol solution, a characteristic absorption peak at 260 nm appeared. The red shift of the UV absorption peak is likely caused by silver complexation with the double bonds of squalene. According to the Dewar–Chatt–Duncanson model, the electrons of unsaturated C=C double bond form a σ coordination bond with the 5 s

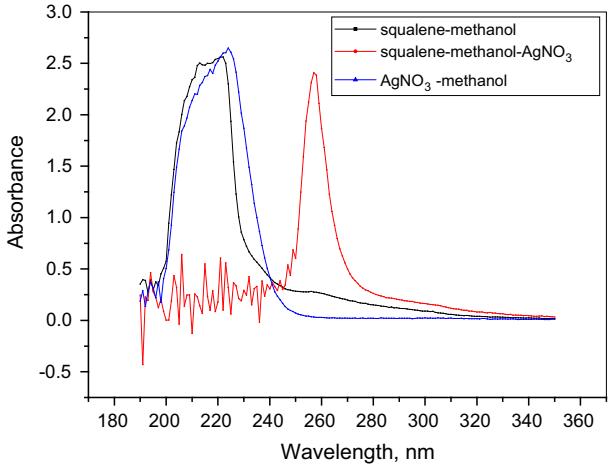

Fig. 1. UV–visible spectra of squalene-methanol (black), squalene-methanol-$AgNO_3$ (red) and methanol-$AgNO_3$ (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)