

Original Research Article

Natural Borneol Recycling from *Cinnamomum camphor* chvar. *Borneol* Oil Residue by Fractional Distillation and Recrystallization

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Abstract

Purpose: To establish an efficient method to recycle natural borneol from *Cinnamomum camphor* chvar. *Borneol* oil residue.

Methods: The fractions and raffinate of the oil residue of *Cinnamomum camphor* chvar. *Borneol* were obtained by fractional distillation, and analyzed by gas chromatography with flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Natural borneol was purified by recrystallization and suction filtration. Fractional distillation and recrystallization parameters, including reflux ratio, solvent and solvent amount, were optimized. The physicochemical properties of natural borneol were determined by melting point and optical rotation techniques.

Results: The total yield of natural borneol was 7.6 % under the optimal conditions, viz, reflux ratios of 3:1 and 1:1, with *n*-hexane as recrystallizing solvent. The purity of recrystallized natural borneol was > 99 % at the solvent ratio of 1:1.8 (natural borneol crystal : *n*-hexane). The optical rotation ($[\alpha]_D^{25} = + 37^\circ$) and melting point (207 °C) of natural borneol were in accord with those of the standard.

Conclusion: These findings indicate that the developed fractional distillation and recrystallization method can be applied to recycle natural borneol from *C. camphor* chvar. *Borneol* oil residue.

Keywords: *Cinnamomum camphora* chvar. *Borneol*, Oil residue, Natural borneol, Fractional distillation, Recrystallization

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INTRODUCTION

Cinnamomum camphora chvar. *Borneol*, a precious and rare medicinal material in Lauraceae family, is discovered in Jiangxi Province and cultivated in many provinces in south China [1]. Due to richest content in natural borneol, the leaves and branches of *C.*

camphora chvar. *Borneol* are industrially applied to extract essential oil for further natural borneol production [2]. Natural borneol (Fig 1) has been widely used in food, medicine, cosmetics, and other industries [3]. To date, extensive efforts have been devoted to the elucidation of chemical components of *C. camphora* chvar. *Borneol* essential oil [4] and its oil residue [5,6]. However,

few of them had ever targeted recycling natural borneol from its oil residue. Steam distillation is widely used in the extraction of essential oil from the leaves and branches of *C. camphora* chvar. *Borneol* in industry [7]. The essential oil was slowly crystallized. After filtration, the pure natural borneol was separated. However, during this process, a mass of oil residue with natural borneol over 23 % was inevitably generated, which is usually to be discarded or merely used as cheap raw materials and spices [5,8]. Hence, oil residue might also be an important source for production of natural borneol. The aim of this study was to investigate the utilization of oil residue for preparation of high-purity natural borneol by virtue of fractional distillation and recrystallization.

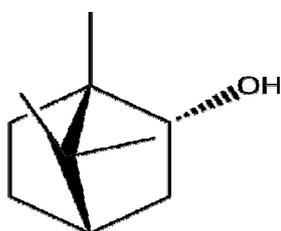


Figure 1: Chemical structure of natural borneol

EXPERIMENTAL

Materials

C. camphora chvar. *Borneol* was collected from the Institute of Forestry Sciences of Ji'an Prefecture of Jiangxi Province (China) in April 2013, and authenticated by Prof. Zi Ren Su at School of Chinese Materia Medica, Guangzhou University of Chinese Medicine, where a voucher specimen (no. 2013122901-09) was deposited in the herbarium. Steam distillation and crystallization were used for the extraction of essential oil from the leaves of *C. camphora* chvar. *Borneol* and production of natural borneol, respectively. The resulting oil residue during this process was employed in the study. Standard borneol was purchased from Sigma-Aldrich Co, Ltd (Shanghai, China). n-hexane and ethyl acetate were from Guangzhou Chemical Reagents Factory, China.

Apparatus

The rectification was carried out with a fractionating tower consisting of a temperature control heating jacket, a round-bottom flask (1000 mL), a distillation column (150 × 25 mm inner Diameter) filled with stainless steel Dixon ring packing (3 × 3 mm inner Diameter), an intelligent thermostat, a reflux condenser and

receiving flasks. The reflux ratio (R) is the ratio of reflux flow (L) to distillate flow (D), measuring the amounts of volatile oil going up the top of the column and returned back to the column as reflux.

Fractional distillation and recrystallization procedure

The oil residue (300 g) was brought to boil in the round-bottom flask of fractionating tower, on condition that the pressure in this system has been kept under normal atmospheric pressure. The oil residue was heated at 170 °C until refluxing, and then maintained at total reflux for 1 h in order to reach vapor-liquid equilibrium condition. The heater was turned on and the temperature in the fractionating column was monitored. Fractions were collected at various boiling point ranges. Then the reflux ratio was increased to 3 (for 2 h) and finally decreased to 1. The experiment should not be stopped until the temperature of the bottom of tower surpassed 210.0 °C. In this experimental protocol, the fractions of oil residue were collected at regular intervals of 20 min, quantified and analyzed by GC-FID and GC-MS respectively. The process flow diagram for fraction distillation and recrystallization of natural borneol from oil residue is shown in Figure 2. According to respective distilled-off temperature, the distillation process generated three fractions: fraction A (containing natural borneol ≤ 1 % by weight), fraction B (natural borneol ≤ 10 % by weight) and raffinate (product fraction). Afterwards, the raffinate was stored and crystallized at 4 °C for 6 d, and then recrystallized. Natural borneol crystals were obtained after suction filtration. The fractional distillation and recrystallization parameters, including reflux ratio, solvent and solvent amount, were optimized.

GC-FID analysis

Quantitative analysis of the fractions were performed by a flame ionization gas chromatography (FID), using Varian-3900 equipment (Varian Corporation, Palo Alto, CA, USA). The column used was a ZB-WAX, 30 m × 0.32 mm ID × 0.25 μm film thickness (Phenomenex Corporation, Torrance, CA, USA); nitrogen was the carrier gas at a flow rate of 1 mL/min; the inlet mode was split 1:20; the injector temperature was 280 °C. The temperature program was as follows: the temperature was initially set at 60 °C, and subsequently increased to 90 °C at 10 °C/min, held isothermal for 5 min, then at 3 °C/min to 160 °C, and then it was raised to 240 °C at

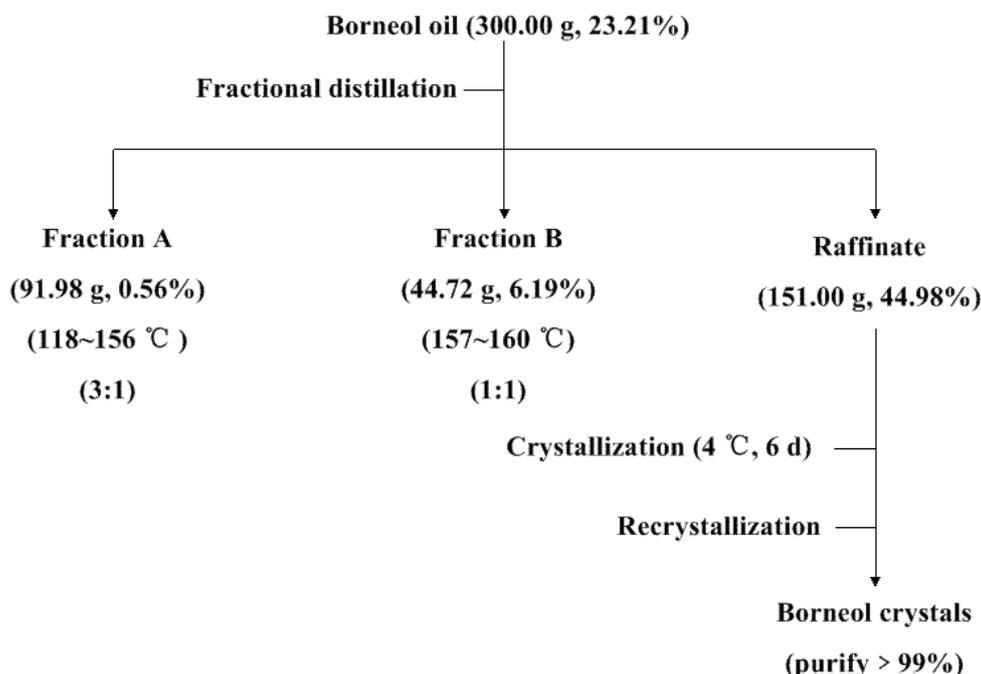


Figure 2: Separation and purification procedure for natural borneol. The yield (weight of oil residue, fraction and crystals, concentration of natural borneol) was shown directly under the fraction, raffinate and crystals. Finally, the crude natural borneol crystals were recrystallized twice to obtain refined natural borneol (purity over 99 %)

30 °C/min. Thereafter, this condition was held for 3 min. The injection volume was 1 μ L. Identification of constituents was based on the retention time compared with the standards under identical experimental conditions.

GC-MS analysis

The GC-MS analysis was performed on an Agilent 6890-5973 model gas chromatograph-mass spectrometer (Agilent Corporation, Santa Clara, CA, USA). The column was equipped with a HP-5MS (crosslinked 5 % Phenyl Methyl Silox) capillary column (30 m \times 250 μ m \times 0.25 μ m film thickness). The operation parameters were as described above for GC-FID, except for using helium as a carrier gas. The GC-MS analysis was conducted at 70 eV with a mass range of 40 - 400 m/z and a scanning velocity of 1494 amu/s. The quadrupole and ion source temperature were 150 and 230 °C, respectively.

Physicochemical properties

One gram natural borneol crystal was dissolved in 10 mL dehydrated alcohol to obtain the final concentration of 0.1 g/mL. The special rotation of the solution was determined with a polarimeter (model 341, Perkin Elmer, USA). Melting point was determined by a melting point apparatus using capillary tubes (model B-545, Büchi, Switzerland).

Statistical analysis

SPSS Statistics 17.0 was used for statistical analysis. The data were presented as mean \pm standard deviation. The statistical significances within a parameter were evaluated by analysis of variance (ANOVA) followed by least significant difference (LSD) test. Values were considered significantly different at $p < 0.05$.

RESULTS

Separation and purification of natural borneol

The weight and natural borneol content of all fractions described above are shown in Figure 2. By fractional distillation, oil residue (300.00 g, 23.21 %) was separated into fraction A (91.98 g, 0.56 %), fraction B (44.72 g, 6.19 %) and raffinate (151.00 g, 44.98 %). Then raffinate was crystallized at 4 °C to obtain crude natural borneol crystals (55.00 g, 73.6 %). Thereafter high-purity natural borneol (23.00 g, 99 %) was obtained by recrystallization. The total yield of natural borneol by fractional distillation and recrystallization was 7.6 %. Natural borneol obtained in this way was white crystalline. Meanwhile, the purified borneol were determined by polarimeter, and the mean optical rotation $[\alpha]_D^{24}$ was + 37 ° (ethanol, 20 °C), which showed that the purified borneol was a dextroisomer. The melting point of natural borneol was 207 °C.

These parameters were fairly in accord with the quality standard of Chinese Pharmacopoeia [9]. From the foregoing results, fractional distillation coupled with recrystallization might be applied to recycle natural borneol from oil residue.

The chemical composition of all fractions

To investigate the process of isolation and purification of natural borneol from oil residue, the chemical compositions of the fractions and natural borneol crystals were analyzed by GC-MS. The total ion current (TIC) chromatograms of oil residue, fraction (A, B and raffinate) and natural borneol crystals are shown in Figure 3. Fraction A was rich in α -pinene (Peak 1) which accounted for the percentage of 22.1 %. Compared with fraction A, the amount of α -pinene decreased to 1.60 % in fraction B, while the purity of camphor (Peak 2) was raised from 0.50 % to 6.23 %, and the content of natural borneol (Peak 3) increased from 0.56 % to 6.19 %. The raffinate (Figure 3D) mainly consisting of natural borneol (44.98 %), bornyl acetate (18.12 %) and camphor (15.33 %) was subjected to crystallization at 4 °C for natural borneol. However, the purity of natural borneol obtained after first crystallization was relatively low. Hence further recrystallization was needed. In the present work, the crude sample of borneol was further purified in n-Hexane. As shown in Figure 3E, results indicated that the purity of natural borneol recrystallized twice was higher than 99 %, hence recrystallization was a favorable choice in the preparation of high-purity borneol.

Recovery of natural borneol in oil residue

Reflux ratio is an important parameter in distillation design and operation. Recovery of

natural borneol and separation time were investigated according to different reflux ratios. The results are given in Table 1. As the reflux ratios increased from 1:1, 2:1 to 3:1, 1:1, the yield and recovery of natural borneol were elevated by 4.5 % ($p < 0.05$) and 10.0 % ($p < 0.05$) respectively. However, with the reflux ratios decreased from 3:1, 7:1 to 3:1, 1:1, yield and recovery declined significantly by 3.0 % ($p < 0.05$) and 6.9 % ($p < 0.05$). It can be seen from these results that the maximum yield (18.3 ± 1.0 %) and recovery (99.4 ± 0.5 %) were obtained at the reflux ratios of 3:1 and 1:1. Total separation time at this reflux ratio was 5.5 h. Considering the effectiveness and power consumption, the reflux ratios of 3:1 and 1:1 were chosen.

The yield (%) of natural borneol in oil residue was the weight of crude crystal to the weight of oil residue. The recovery (%) was the concentration of natural borneol in raffinate to that in C. camphora chvar. Borneol oil residue. Treatment meant with the same letter was not significantly different from each other at $p > 0.05$.

Recrystallization yield and recovery of natural borneol

The selection of an appropriate solvent is of vital importance in the recrystallization process. In order to investigate which solvent was more suitable, ethyl acetate and n-hexane were chosen. In this experiment, 1 g crude natural borneol were dissolved in 2 mL ethyl acetate and 3 mL n-hexane to obtain high purity natural borneol by recrystallization, respectively. The results are shown in Table 2. It was suggested that the purity, yield and recovery of natural

Table 1: Effects of different reflux ratios on yield and recovery of natural borneol in oil residue

Reflux ratio	Separation time (h)	Yield (%)	Recovery (%)
1:1 ^A , 2:1 ^B	5.0	13.8 ± 0.5 ^a	89.4 ± 1.2 ^a
3:1,7:1	8.5	15.3 ± 1.1 ^b	92.5 ± 1.5 ^b
3:1,1:1	5.5	18.3 ± 1.0 ^c	99.4 ± 0.5 ^c
5:1,3:1	7.0	16.9 ± 0.5 ^{bc}	95.7 ± 1.4 ^d

Note: ^A The reflux ratio as fraction A distilled off; ^B The reflux ratio as fraction B distilled off

Table 2: Recrystallization of natural borneol in ethyl acetate and n-hexane

Solvent	Material/solvent ratio	Contents (%)	Yield (%)	Recovery (%)
Ethyl acetate	1:2	83.4 ± 0.6 ^a	67.2 ± 1.8 ^a	76.1 ± 1.4 ^a
n-Hexane	1:3	85.9 ± 1.3 ^b	76.5 ± 1.3 ^b	89.3 ± 1.2 ^b

Note: Material/solvent ratio is the ratio required to dissolve the crude natural borneol

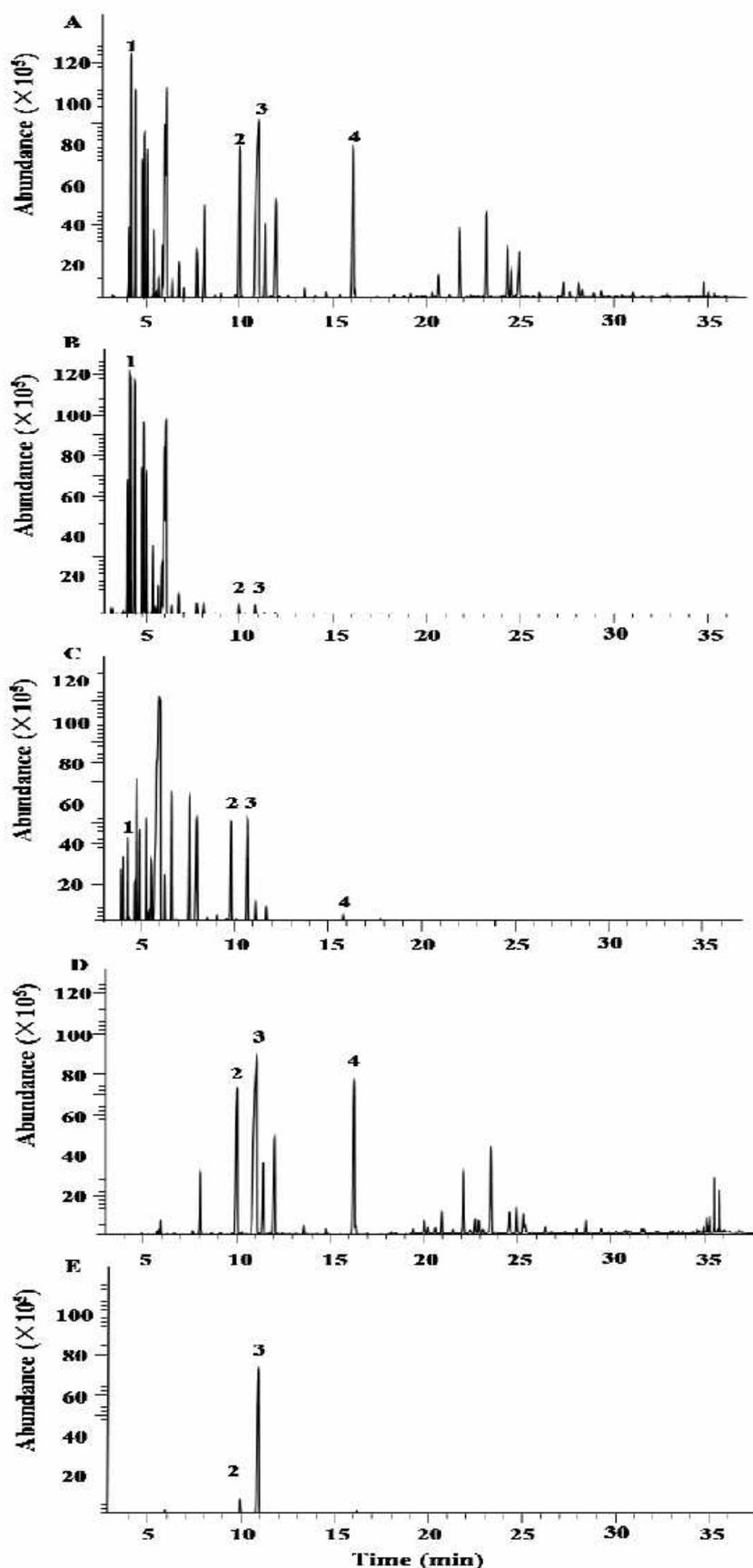


Figure 3: Typical GC-MS chromatograms: (A) oil residue, (B) fraction A, (C) fraction B, (D) raffinate; (E) purified natural borneol. Peak identification: (1) α -pinene, (2) camphor, (3) natural borneol, (4) bornyl acetate

borneol crystallized from n-hexane were higher than that from ethyl acetate ($p < 0.05$). Consequently, n-hexane might be superior to ethyl acetate as the recrystallization solvent for natural borneol purification.

The yield (%) of natural borneol was the weight of refined natural borneol to the crude natural borneol. The recovery (%) was the concentration of natural borneol in refined crystals to the concentration of crude natural borneol. Treatment meant with the same letter was not significantly different from each other at $p > 0.05$.

Effect of solvent on natural borneol crystallizing

In order to find the favorable n-hexane ratios for natural borneol crystallizing, five different ratios namely 1:1.2, 1:1.5, 1:1.8, 1:2.0 and 1:2.2 were investigated (Table 3). The results showed that the content of natural borneol was elevated and the yield and recovery declined when n-hexane ratios rose from 1:1.2 to 1:2.2. As the n-hexane ratio increased from 1:1.8 to 1:2.2, the yield and recovery decreased obviously ($p < 0.05$). However, the purity of natural borneol was elevated only by 0.5 % ($p > 0.05$). Considering the treatment effect and solvent consumption, n-Hexane ratio of 1:1.8 was chosen as the optimum solvent for recrystallizing natural borneol.

DISCUSSION

According to the Chinese Pharmacopoeia, borneol is an ingredient of over 60 herbal products [9]. It is widely accepted that synthetic borneol is more toxic than natural borneol. Therefore, a strategy to avoid the toxic effects is to replace synthetic borneol with natural borneol in different borneol products, and the new sources for natural borneol would provide this opportunity [10]. The essential oil obtained by hydrodistillation of young leaves of *C. camphora* chvar. *Borneol* was richest in natural borneol with content over 66.8 % [11]. The essential oil derived from the leaves was subjected to crystallization. After filtration, the pure natural borneol was separated from oil residue in this process. Reports have revealed that the content of natural borneol in oil residue was still over 23 % and deserved further exploration [5,8]. In this work, fractional distillation combined with recrystallization allowed natural borneol to be obtained with the yield 7.6 % and purity 99 % from oil residue. Moreover, natural borneol product obtained by this method met the quality standard of the Chinese Pharmacopoeia [9].

Therefore, the established regime might serve as an effective alternative for the production of high-purity natural borneol.

Fractional distillation, is a highly efficient and solvent-free separation process which depends on the differences of components' boiling points. As shown in the total ion current (TIC) chromatogram of oil residue (Figure 3A), components of oil residue such as α -pinene, limonene and linalool were lower than that of natural borneol. Consequently, these components could be distilled prior to natural borneol, divided into fraction A and B, and then the raffinate consisting mainly of natural borneol obtained.

However, as TIC chromatogram displayed, there were some component such as camphor (Peak 2) which had similar molecular weights and boiling points as natural borneol difficult to be separated by fractional distillation. Column chromatography method was previously applied to isolate natural borneol, however, the process consumed large amount of solvents and the yield and recovery were also low [8]. In this work, recrystallization of crude natural borneol crystal was carried out with n-hexane, and natural borneol was achieved with purity over 99 %.

Reflux ratio is a crucial parameter in distillation design and operation, which has an important impact on the separation effect and the distillation cost [12]. The purity of target constituent can be improved by increasing the reflux ratio when quantity of column plate is fixed. However, the increasing reflux ratio will push up the energy cost [13]. In this study, the appropriate reflux ratio was investigated. As the reflux ratio increased, more liquid rich in more volatile components was recycled back into the column, and the energy consumption was in positive correlation with the separation time. Results showed that the yield and recovery of natural borneol in raffinate were higher and the separation time was shorter, when fraction A and fraction B were distilled off at the reflux ratios of 3:1 and 1:1, respectively.

The yield was about 3 % and was higher than that at reflux ratios of 3:1 and 7:1, indicating that the yield increased as reflux ratio decreased. This might be associated with the dehydrogenation from natural borneol to camphor under long-time high temperature heat. However, precise mechanism deserved further investigation. Natural borneol represented 44.98 % of the raffinate after reflux. The relatively higher concentration of the target compound in raffinate reduced the difficulty in further process.

Table 3: First recrystallization of natural borneol in various solvent ratios

<i>n</i> -Hexane ratio	Content (%)	Yield (%)	Recovery (%)
1:1.2	85.9 ± 0.8 ^a	76.5 ± 0.9 ^a	89.3 ± 0.5 ^a
1:1.5	89.3 ± 1.0 ^b	69.1 ± 1.0 ^b	83.8 ± 0.6 ^b
1:1.8	93.8 ± 1.2 ^c	65.6 ± 0.6 ^c	83.6 ± 0.3 ^b
1:2.0	94.1 ± 1.6 ^c	58.1 ± 0.8 ^d	74.3 ± 1.0 ^c
1:2.2	94.3 ± 0.6 ^c	50.0 ± 1.0 ^e	64.1 ± 0.9 ^d

Treatments with the same superscript letter were not significantly different from each other at $p > 0.05$

Based on the experimental results, the reflux ratios of 3:1 and 1:1 were chosen in order to obtain the highest yields of natural borneol.

The selection of a suitable solvent is a critical part of the recrystallization procedure [14]. Natural borneol is a small lipophilic compound and freely soluble in ethanol, chloroform and ethyl acetate. Song et al. [6] used gasoline to crystallize natural borneol with the recovery 68 %. He et al [8] applied gasoline and ethyl acetate to purify natural borneol, and the recoveries were 70 % and 57 %, respectively. *n*-Hexane is a major constituent of gasoline, and widely used as cheap and relatively safe solvent in the extraction and refining process. However, there is no report on the purification of natural borneol using *n*-hexane as recrystallization solvent. Therefore, *n*-hexane and ethyl acetate were investigated in this work. The crude crystals were fairly soluble in the boiling *n*-hexane and only sparingly soluble in *n*-hexane at room temperature. However, impurities were quite soluble in the solvent at room temperature. In this way, when the solvent was cooled and the natural borneol crystallized out, any soluble impurities remained were removed by filtration. Based on the results obtained, *n*-hexane might be a more appropriate solvent for crystallizing natural borneol from *C. camphora* chvar. *Borneol* oil residue due to higher purity, yield and recovery.

Solvent amount is another factor affecting recrystallization efficiency [15]. As showed in Table 2, the content of natural borneol increased from 85.9 ± 0.8 % to 94.3 ± 0.6 %, with respect to the *n*-hexane ratio of 1.2 to 2.2. When the *n*-hexane ratio was 1:1.8, the purity of natural borneol was 93.8 ± 1.2 %, and the yield and recovery were 65.6 ± 0.6 % and 83.6 ± 0.3 %, respectively. However, as the *n*-hexane ratio increased from 1:1.8 to 1:2.2, the yield and recovery decreased significantly ($p < 0.05$). Hence, the *n*-hexane ratio of 1:1.8 was employed.

CONCLUSION

Fractional distillation followed by recrystallization is an efficient method for recycling natural borneol from *Cinnamomum camphor* chvar. *Borneol* oil residue. The proposed method is economical and practical, in terms of resource optimization, with relatively higher purity, yield and recovery. Hence, it is potentially an effective option for the production of high-purity natural borneol and should also suitable for industrial scale-up.

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