Purification of ricinoleic acid methyl ester using mesoporous calcium silicate (CaSiO₃) adsorben

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Abstract. Ricinoleic acid methyl ester has been purified from a mixture of fatty acid methyl esters resulted from transesterification of castor oil using a 200 mesh mesoporous calcium silicate adsorbent. The purity of ricinoleic acid methyl ester was increased from 48% to 94% conformed by gas chromatography. It is proposed that in adsorption process, a strong interaction between calcium silicate and ricinoleic acid methyl ester occurs through empty d-orbital of Ca-atom of calcium silicate and O-atom of the hydroxyl group of ricinoleic acid methyl ester. The resulting ricinoleic acid methyl ester was confirmed by ¹H-NMR and ¹³C-NMR.

1. Introduction

Recently, research in polar absorbent nature of non transition metals particularly alkaline earth metals such as Mg, Ca, Sr or Ba has been more intriguing. The alkaline earth metal salts, M-PSS and MSiO₃ (M = Mg, Ca., Sr or Ba; PSS = polystyrene sulfonate), have been used as adsorbents in absorption of organic matter such as fatty acids, carotenoids from crude palm oil (CPO), and tocoferol and tocotrienol from candle nut oil [1, 2, 3]. M-PSS have been used to absorb carotenoids from CPO followed by desorption of the carotenoids from the adsorbents [1, 2]. It was reported that in adsorption and desorption processes, the alkaline earth metals played an important role in the interaction of the metals with the carotenoids. The M-PSS absorbents could adsorb 77% - 85% of carotenoids and desorp them in 32% - 84% and the highest recovery of carotenoid showed by Ca-PSS which was 65% [1, 2]. Similarly, Sebayang et.al. used CaSiO₃ and Ca-PSS to enrich tocopherol and tocotrienol from candle nut oil. Using CaSiO₃ tocopherol could be enriched from 0.24% to 5.52% and tocotrienol from 0.22% to 4.23%, while using Ca-PSS tocopherol could be enriched from 0.24% to 6.39% and tocotrienol from 0.22% to 4.96% [3]. Masmur et.al. have also studied the interaction of empty d-orbitals with σ-electron donor atoms of free fatty acids (FFA) from CPO by using polar MSiO₃ (M = Mg, Ca, Sr or Ba) adsorbents. The FFA contents in CPO were reduced up to 78% - 90%, with the highest FFA adsorbed was 90% [4]. From all alkaline earth adsorbents used above, calcium salts showed the best result.

Ricinoleic acid methyl ester (RAME) is an important material which is used in a wide range of application such as a row pharmaceutical material for production of conjugated linoleic acid (CLA) [5,6,7], as disinfectant for injury, laxative agent and for renewable polyester [8, 9]. Methyl esters resulted from transesterification of castor oil were always as a mixture of fatty acid methyl esters (FAME) with composition of methyl ricinoleate 85%, methyl palmitate 1.4%, methyl stearate 1%, methyl oleate (3.4%, methyl linoleate 5.7%, linolenate 0.6%) [10]. Several works have been
accomplished to purify RAME, mostly by distillation or by liquid-liquid extraction which may take a long time and sequences of works [10, 11].

We have attempted to use CaSiO₃ adsorbent to purify RAME from a mixture of FAME resulted from transesterification of castor oil. RAME through its hydroxyl group can make a strong interaction with empty d-orbital of Ca-atom of CaSiO₃ while the other fatty acid esters can only make weaker interactions via their double bonds and ester groups. Here we report our finding about effect of empty d-orbital of CaSiO₃ adsorbent in purification of RAME.

2. Materials and methods

2.1 Materials

Methanol, ethanol, n-hexana, potassium hydroxide, calcium chloride dan water glass were purchased from E-merk. Ricinus communis seeds as a source of castor oil was obtained from local farmer (around Medan). The castor oil was extracted at reflux for 6 hours from oven dried Ricinus communis seeds with an equal volume of a mixture of ethanol and n-hexane. FAME were obtained by transesterification of the castor oil which was conducted in a dry methanol using potassium hydroxide as a catalyst. The composition of methyl esters was determined by Gas Chromatography Agilent MS 7890B or Agilent MS 5977B, using HP-5MS column, with 30 m long, 0.25 mm in diameter and 0.25 µm film. FT-IR spectra were recorded by Shimadzu 440. 1H-NMR dan 13C-NMR spectra were recorded by Agilent 500 MHz spectrometer. Chemical shift for proton were reported in ppm relative to TMS as an external standard. Mesoporous calcium silicate adsorbent (200 mesh) with a pore size of 5,585 nm was prepared as reported in reference [2].

2.2 Transesterification of Castor Oil

FAME were obtained from the reaction of castor oil (40.00 g, 0.13 mol) and dry methanol (85.76 g; 2.68 mol) in the presence of KOH (0.8 g, 0.014 mol), refluxed for 4 hours. After the reaction, excess KOH was dissolved with hot water followed by extraction of the esters with hexane. Removing the hexane gave a mixture of fatty acid methyl esters (30 g, 72.5%). The composition of methyl esters was determined by GC and FT-IR.

2.3 Purification of RAME

RAME was purified from the mixture of FAME obtained from transesterification of castor oil. It was adsorbed by CaSiO₃ in a small chromatographic column (h = 50 cm; d = 0.5 cm). The column was filled with a 200 mesh CaSiO₃ absorbent (1 g, h = 10 cm) and a solution of FAME (1.2 mL) in hexane (10 mL) was added to the column then the esters mixture was allowed to stand for 2 h. The FAME were then eluted with hexane to remove all the fatty acid methyl esters from the CaSiO₃ adsorbent, but the RAME remain tightened in the adsorbent. The RAME was then freed from the adsorbent by a polar solvent, ethanol. Removing ethanol gave RAME (0.5 mL, 87%). The resulting RAME was characterized by GC (only one peak appears), 1H-NMR (CDCl₃) δ 5.46 and 5.35 ppm (-CH=CH-), δ 3.57 ppm (OCH₃), δ 3.53 ppm (-CHOH-C), δ 2.22 ppm (-CH₂-COOCH₃), δ 2.19 ppm (OH), δ 2.12 ppm and δ 2.10 ppm (-CH₂CH=CH-CH₂-), δ 1.54 – 1.17 ppm (rest of -CH₃-) and δ 0.8 ppm (CH₂CH₃) and 13C-NMR (CDCl₃) δ 174.2 ppm (-COOCH₃), δ 132.8 ppm and δ 125.3 ppm (-CH=CH-), δ 71.3 ppm (C=OH), δ 36.7 - 22.5 ppm (rest of -CH₂-) and δ 14 ppm (-CH₃).

3. Results and discussion

3.1 Transesterification of castor oil

The castor oil was obtained by extraction from oven dried Ricinus communis seeds. The castor oil was then transesterified with methanol producing a mixture of FAME. The composition of FAME in the mixture was determined by gas chromatography and the result is shown in Table 1.
Table 1 The composition of methyl ester from castor oil

| No. | Name of Compounds          | Percentage (%) |
|-----|----------------------------|----------------|
| 1.  | Palmitic acid methyl ester | 4.17           |
| 2.  | Ricinoleic acid methyl ester | 48.76         |
| 3.  | Stearic acid methyl ester  | 6.55           |
| 4.  | Linoleic acid methyl ester | 23.53          |
| 5.  | Oleic acid methyl ester    | 1.73           |
| 6.  | Others                     | 15.26          |

The FT-IR spectrum of FAME is shown in Figure 1. A sharp strong peak at 2801 cm\(^{-1}\) was assigned to the CH vibration frequency of the hydrocarbon chain of the methyl esters, which is shifted 52 cm\(^{-1}\) from that of castor oil which appears at 2853 cm\(^{-1}\) [12]. Another strong peak appears at 1744 cm\(^{-1}\) is assigned to the presence of the carbonyl group of the methyl esters.

![FT-IR spectrum of fatty acid methyl ester from transesterification of castor oil](image)

Figure 1 FT-IR spectrum of fatty acid methyl ester from transesterification of castor oil

3.2 Purification of RAME from FAME

Methanol transesterification of castor oil gave a mixture of FAME. In order to purify RAME, a solution of FAME in hexane was added to a chromatographic column that had already been filled with a 200 mesh CaSiO\(_3\) adsorbent. The solution of FAME was allowed to stand for 2 hours in the column to complete the adsorption of RAME. After 2 hours, the adsorbed esters in the column were desorbed by eluting them with excess of hexane. In this process all non-hydroxyl methyl esters were removed from the column while RAME remain attached to the CaSiO\(_3\) adsorbent. Although all the fatty acid esters have partial polar groups, they were not interact as strong as the interaction of the hydroxyl group of RAME to the absorbent. High solubility of the esters in hexane causing the miscibility among the esters, due to nonpolar-nonpolar interactions among them, could be broken so that the esters could be removed using an excess amount of hexane. The presence of hydroxyl group in RAME has increased its polarity causing a stronger interaction with adsorbent where a nonpolar solvent like hexane would not be able to break the bond and it will remain attached in adsorbent. RAME can be desorbed from CaSiO\(_3\) adsorbent by eluting it with a polar solvent ethanol. Removing ethanol gave
RAME in a good yield, with a purity of ca. 94% determined by gas chromatography. The chromatograms of FAME before and after adsorption by CaSiO$_3$ adsorbent are shown in Figure 2.

Figure 2 The chromatograms of FAME. a. Before adsorption b. After adsorption

In chromatogram a, it is clearly seen that before adsorption by CaSiO$_3$, there are 5 peaks present due to the presence of 5 methyl esters in the mixture of FAME as shown in Table 1, and after adsorption of FAME only one peak appears with a retention time of 12.6 minutes, showing the presence of RAME as is shown in chromatogram b. This result confirms that interaction between RAME and CaSiO$_3$ adsorbent is stronger than interactions of other FAME with CaSiO$_3$. The interaction between RAME and CaSiO$_3$ adsorbent is proposed by using O-atom of the hydroxyl group of RAME and the empty d-orbital of Ca-atom as shown in Figure 3. The oxygen atom of the hydroxyl group of RAME which is a $\sigma$-donor electron can distribute some electron density to empty d-orbital of Ca-atom which is an electron acceptor.

In order to confirm that RAME has been isolated from the mixture of FAME, the liquid that was obtained from chromatographic column was then further characterised by $^1$H-NMR and $^{13}$C-NMR. The $^1$H-NMR spectrum of RAME is clean as is shown in Figure 4. The spectrum shows the appearance of peaks at $\delta$ 5.46 and $\delta$ 5.35 ppm due to the presence of two alilic double bond protons, -CH=CH-, while the peak at $\delta$ 3.57 ppm is assigned to the methoxy protons, OCH$_3$, and the peak at
δ 3.53 ppm is given by the proton of -CHOH-C. The peak at δ 2.22 ppm is assigned to the protons adjacent to the ester group, -CH₂-COOCH₃, and at δ 2.19 ppm is given by the hydroxyl proton, OH while the peaks at δ 2.12 ppm and δ 2.10 ppm are assigned to the protons adjacent to the double bond, -CH₂CH=CH-CH₂-. The peaks at δ 1.54 - 1.17 ppm are assigned to the rest of -CH₃ protons in the hydrocarbon chain and the peak at δ 0.8 ppm is given by the methyl protons, -CH₃CH₃.

Further confirmation of RAME was done by running its ¹³C-NMR. The ¹³C-NMR spectrum of RAME is shown in Figure 5. The presence of the ester carbonyl group is shown by the appearance of a peak at δ 174.2 ppm, while the peaks at δ 132.8 ppm and δ 125.3 ppm are assigned to the carbon double bond, -CH₂=CH₂-. At δ 71.3 ppm is appeared a peak due to the carbon attached to the hydroxyl group, C-OH and the peak at δ 51.3 ppm is given by the carbon of the methoxy group, OCH₃. All the peaks that appear at δ 36.7 - 22.5 ppm are assigned to the rest of methylene group, -CH₂-, in hydrocarbon chain, while the peak at δ 14.0 ppm is given by the methyl group, -CH₃ at the end of the chain. Both ¹H-NMR and ¹³C-NMR spectra are in a good agreement to confirm that RAME has been isolated.
4. Conclusions
Ricinoleic acid methyl ester can be isolated from a mixture of fatty acid methyl esters, obtained from transesterification of castor oil, using a 200 mesh calcium silicate adsorbent with a purity of 94%. The presence of hydroxyl group in ricinoleic acid methyl ester and the availability of the empty d-orbital of the Ca-atom in calcium silicate adsorbent resulted in a strong interaction between them. On the other hand, the other non-hydroxyl fatty acid methyl esters in the mixture can only make a weaker interaction with the adsorbent that can easily be broken by eluating them with nonpolar hexane solvent, leaving ricinoleic acid methyl ester attached to the adsorbent, so that they will be removed from the adsorbent. The isolated ricinoleic acid methyl ester with a purity of 94% was confirmed by gas chromatography, $^1$H-NMR and $^{13}$C-NMR spectroscopy.

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