Enrichment of Tocopherol and Tocotrienol using Polar Adsorbents from Candlenut Oil

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Abstract—***Polar adsorbents have been used to enriched tocopherol (TP) and tocotrienol (T3) in adsorption and desorption methods from candlenut oil. The adsorbents were Calcium Polystyrene Sulfonate (Ca-PSS), Aluminium Polystyrene Sulfonate (Al-PSS), Calcium Methyl Ester Sulfonate (Ca-MES) and Calcium Silicate (Ca-S). Column mode was carried out by passing through the candlenut oil with each of adsorbents. To recover the TP and T3 which were adsorbed, n-hexane and toluene were used then the TP and T3 that have been extracted were control by HPLC. Ca-PSS show the highest enrichment of TP and T3 from candlenut oil which were 6.39% (0.0104 g) and 4.96% (0.0081 g) respectively from the initial TP and T3 were 0.24% (0.0012 g) and 0.22% (0.0011 g) respectively.***

Keywords—**Tocopherol, Tocotrienol, Adsorption, Extraction, Polar adsorbent**

I. INTRODUCTION

Tocopherols (TP) and Tocotrienols (T3) which known as tocochromanols, are isomer group of vitamin E. Vitamin E play a role of nutrition for human body. Tolerable upper intake level (UL) of vitamin E which is set up by Scientific Committee on Food (SCF) for adults of 300 mg α-tocopherols per day. JECFA has defined an Acceptable Daily Intake (ADI) of 0.15-2 mg/kg/day [1].

Tocopherols have a phytyl tail which contains a saturate C_{16} side chain while tocotrienols contains three trans double bonds. Based on the position of methyl groups, both tocopherols and tocotrienols have four main constituents which are alpha, beta, gamma and delta [2]. Tocopherols and tocotrienols occur in plants in variable amounts, and have varies of biological and antioxidant activity [3]. Tocopherols and tocotrienols can be found in candlenut oil which is 59.9 ppm and 129.3 ppm respectively [4]. Candlenut oil is essential for human and found as cooking ingredient in Indonesia [5]. Major component of candlenut oil is triglyceride and the minor components are tocopherols and tocotrienols. Both components are very soluble in triglyceride. Some reported, has been attempted to separate both minor components.

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Adsorption methods using polymer diaion HP 20, sepabeads, silica gel, aluminium oxide, synthetic brominated polyaromatic SP 207 and dowex optipore L-285 have been reported to separated tocopherols and tocotrienols from palm oil [6-7].

Sulfonated polystyrene salt has been reported as an adsorbent, shown that have a good ability to adsorb carotenoids. Recently reported Ca-PSS with a degree of sulfonation (DS) 9.1% has demonstrated on adsorption carotenoids of CPO. This adsorbent has high activity but poor to release carotenoids in desorption process. It may be due to low degree sulfonation so low polarity of the polymer, therefore strong adsorption to non-polar component [8-9]. The higher sulfonation degrees is resulting the more ionic polymer properties. It means higher solubility polymer in water [10].

Using the higher polarity of the polymer will affect the triglyceride through ester link and weaker attract to TP and T3. Adsorbents and solvents are affected in the process desorption of TP and T3.

In this study, four types of adsorbents were used they are calcium polystyrene sulfonate, aluminium polystyrene sulfonate, calcium methyl ester sulfonate and calcium silicate. Due to stability of tocopherols and tocotrienols, the solvent used as desorption solvent was *n*-hexane and toluene. As a hydrophobic solvent, toluene has been used to stabilize carotenoids in extraction process [11-13].

II. MATERIALS

Candlenut, Crude palm oil (CPO) was received from PTPN II Tanjung Morawa, Medan, Indonesia, $AlCl_3$, $CaCl_2.2H_2O$, NaOH, *n*-hexane, toluene and ethanol was purchased by E-merck. Methyl Ester Sulfonate (MES) was received from Wilmar, Indonesia. Ca-S was prepared by Ref [8] (Ca-S pore size was 5.585 nm) while Al-PSS (content of Al was 2.59%) and Ca-PSS (content of Ca was 7.9%) were prepared by Ref [9] with sulfonation degree 32.06% (determined by titration).

Content of tocopherols and tocotrienols were determined by HPLC with column type EC 250/4 nucleosil 100-5 with *n*-heptane: ethyl acetate (97:3) as a mobile solvent.

III. METHODS

A. *Preparation of Calcium Methyl Ester Sulfonate (Ca-MES)*

MES was prepared as aqueous solution then added by NaOH 30% then stirred until pH ≤ 5. After evaporated the solvent, the resulting white powder was washed with ethanol, dried and Na-MES has been form. Na-MES then prepared as aqueous solution then added by $CaCl_2$ 30% (1:2 mol) and stirred. The solvent were evaporated then the white powder that obtained was washed with ethanol, dried and weight to result Ca-MES. Sulfonation degree of Ca-MES was 22%.

B. Extraction of candlenut oil from candlenut seeds

Candlenut was blend with *n*-hexane then the mixture going to maceration process for two days. After maceration process, the mixture was separate. The filtrate was separated from solvent under vacuum distillation and then the oil obtained was determined TP and T3 content by HPLC. That found 0.24% (0.0012 g) of TP and 0.22% (0.0011 g) of T3, the rest assume as triglyceride.

C. Adsorption/desorption of tocopherols and tocotrienols using adsorbants Ca-PSS, Al-PSS, Ca-MES and Ca-S from Candlenut Oil.

0.25 g candlenut oil was passed through a column (diameters 3 mm) containing 0.5 g Ca-PSS. 2 ml of a mixture *n*-hexane and toluene were injected to the column. After a few minutes, a yellow liquid dropped out and was collected in a flask. The obtained liquid was dried in vacuum until free from solvent then weight (*dry product*). The product, yellow oily liquid, was analyzed TP and T3 by HPLC.

The same procedures were carried out for variation weight 0.5 g, 1 g, 1.5 g.

The similar work was done using Al-PSS and Ca-MES as adsorbent without weight variation.

To know the effect of toluene, the similar work was done using Ca-PSS without using toluene and repeated for weight variation. Then, another procedure carried out with toluene variation from 1 ml to 3 ml.

Then, Ca-PSS and Ca-S were carried out as adsorbents with weight variation of candlenut oil using *n*-hexane as desorption solvent while toluene was already set up in a flask.

IV. RESULT AND DISCUSSION

Adsorption/desorption of TP and T3 using Ca-PSS from 0.25 g candlenut oil with variation of adsorbent weight was showed in Figure 1.

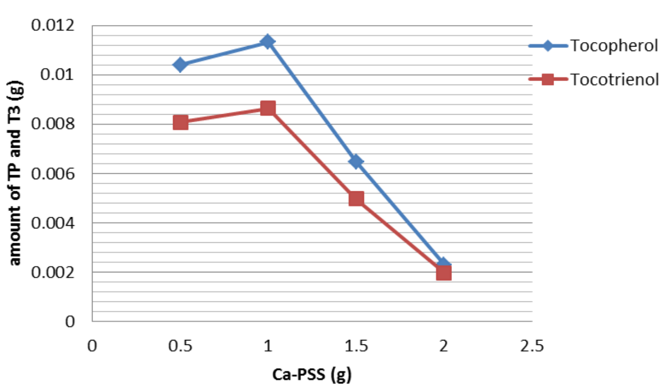


Fig. 1. Amount of TP and T3 (g) obtain after adsorption using Ca-PSS adsorbent with adsorbent weight variation

From Figure 1 show that the maximum amount of Ca-PSS used to adsorbs/desorbs TP and T3 from 0.25 g candlenut oil was 0.5 g with ratio 1:2. TP and T3 enriched for 26 times and 22 times respectively.

To compare this result with another sulfonated polymer salts, Al-PSS carried out to adsorbs TP and T3. Aluminum oxide has been used as adsorbent and has a good capability to adsorb TP and T3 [7, 11]. For this reason, aluminium had been chosen as metal ion of polystyrene sulfonate. Amount of TP and T3 that adsorbs by Al-PSS shown at Figure 2.

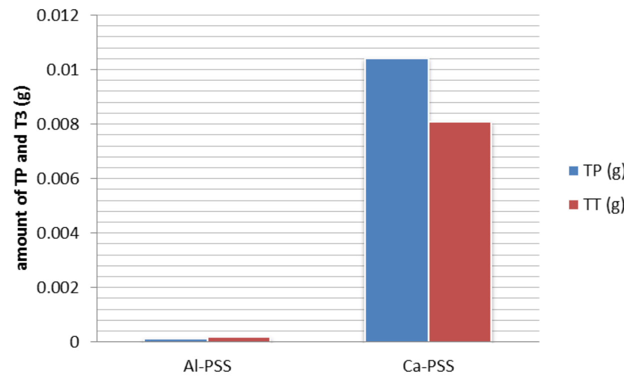


Fig. 2. Comparison of TP and T3 (g) obtain after adsorption/desorption using Al-PSS and Ca-PSS

Figure 2 expressed that amount of TP and T3 that obtain by using Al-PSS were poor. Metal ion which was binding polystyrene sulfonate also involved in the adsorbent capacity to adsorbing TP and T3. The used of calcium as a metal ion enhance interaction of TP and T3. The interaction of calcium metal and aluminium metal with TP and T3 were different, depends on empty orbitals that owned.

This result also happened because of the metal content in sulfonated polystyrene salts. Aluminium content in Al-PSS was only 2.59% while calcium content in Ca-PSS was 7.9%. Ca-PSS has the larger adsorption capacities than Al-PSS.

With this large capacity, Ca-PSS also adsorbs major components such as triglycerides and tends to hold triglycerides at the desorption process then release TP and T3.

Other sulfonate has been carried out to compare the result of Figure 1. With the same metal ion, sulfonated methyl ester and sulfonated polymer had compare to obtain TP and T3 that show at Figure 3.

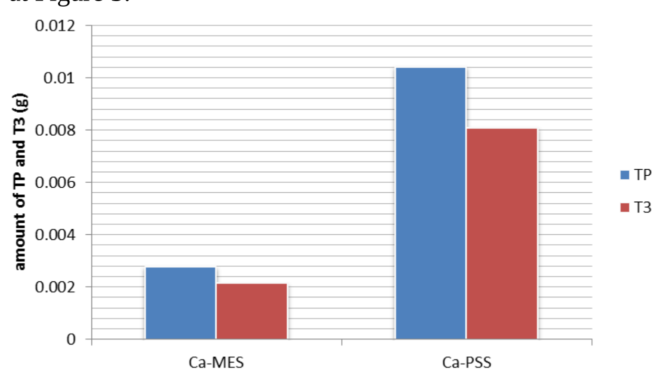


Fig. 3. Comparison of TP and T3 (g) obtain after adsorption/desorption using Ca-MES and Ca-PSS

Ca-PSS show higher result of TP and T3 than Ca-MES. The polarity of polystyrene sulfonate increased if the sulfonation degree higher than 30% [10]. While polystyrene sulfonate shows 32.06% while methyl ester sulfonate shows 22%. Because of more polarity of Ca-PSS, it was easier to release TP and T3. While Ca-MES show less polarity if compared with Ca-PSS. Because of its less polarity properties, Ca-MES tends to hold TP and T3 stronger than triglyceride so the amount of TP and T3 were less than using Ca-PSS.

Effect of toluene

Another method carried out to know the role of toluene to stabilize TP and T3. Figure 4 show desorption method without toluene as solvent using Ca-PSS as adsorbent with weight variation of Ca-PSS.

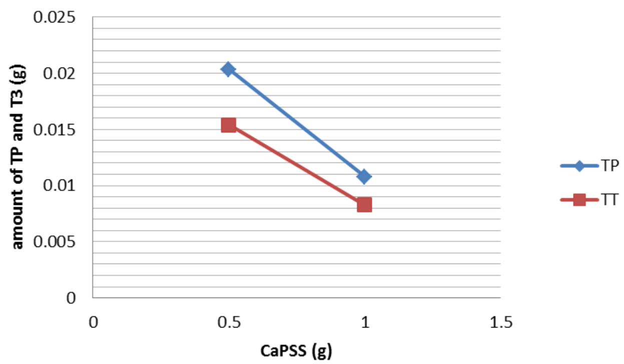


Fig. 4. Amount of TP and T3 (g) obtain after adsorption/desorption without using toluene as solvent

From Figure 4 show that TP and T3 obtain decrease with increasing the amount of Ca-PSS were used even the ratio was 1:2. Without toluene, the amount of TP and T3 were decrease significantly.

While Figure 5 show a desorption condition with toluene variation. Total of solvent used in this method were 5 ml.

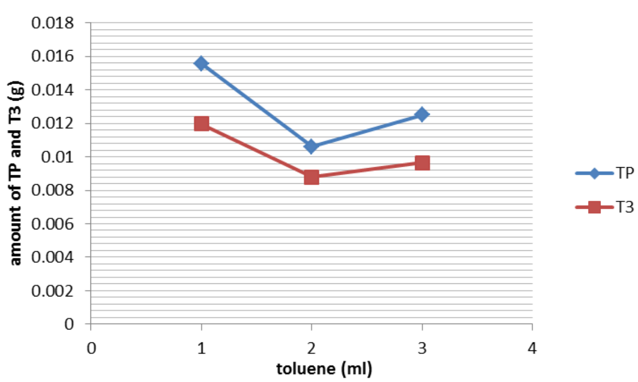
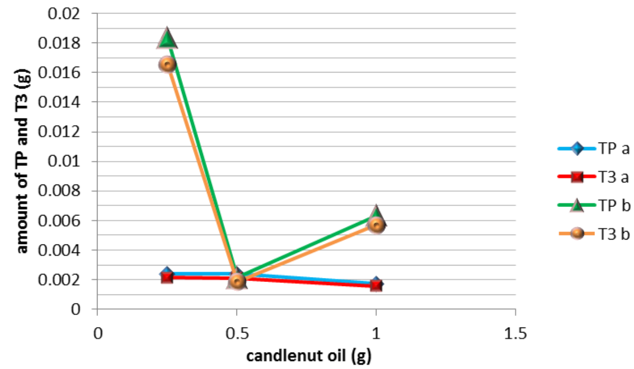


Fig. 5. Amount of TP and T3 (g) obtain with toluene variation

Highest amount of TP and T3 obtained from Figure 5 using 1 ml toluene from 5 ml solvent.

Changing methods

To extend the effect of toluene that used, another method was used to result highest amount of TP and T3. Toluene was set up in flask while *n*-hexane was used as desorption solvent. Ca-PSS (a) and Ca-S (b) were carried out as adsorbent for this method. Amount of TP and T3 which obtain shown at Figure 6.



Note: TP a = amount of tocopherols obtain by using Ca-PSS
T3 a = amount of tocotrienols obtain by using Ca-PSS
TP b = amount of tocopherols obtain by using Ca-S
T3 b = amount of tocotrienols obtain by using Ca-S

Fig. 6. Amount of TP and T3 (g) obtain with toluene in flask using Ca-PSS and Ca-S as adsorbent

Calcium silicate was polar adsorbent indicate with the silanol groups [15]. Even though Ca-S was polar adsorbent, Ca-S shows fewer amounts of TP and T3 that obtained after adsorption/desorption processes. With large surface area, Ca-S adsorbs triglyceride stronger than TP and T3. Carrying out *n*-hexane as a solvent tends to holding on TP and T3 stronger due amount of TP and T3 less then triglyceride. While, *n*-hexane is non-polar solvent that has ability tend to dissolve non-polar compound. When *n*-hexane was passing by the column, triglyceride was release before TP and T3.

A little amount of *n*-hexane that used in this method induces lack of *n*-hexane to dissolve TP and T3.

When the amount of Ca-S increased, the amount of TP and T3 that obtained were fewer.

V. CONCLUSIONS

The enrichment of TP and T3 using four polar adsorbents under Ca-PSS as adsorbent gives the highest performance that increasing 26 times (6.39%) of TP and 22 times (4.96%) of T3 obtained from candlenut oil with using toluene as co-solvent.

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