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***Research paper***

# Extraction And Characterization Of Policosanol From Wheat Germ

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**Abstract**

Policosanol is a mixture of long chain alcohols, with chain lengths varying between 20 to 36 carbon atoms that has been proven to be effective in lowering cholesterol levels in the body. The objective of this research is extraction the policosanol from wheat germ and characterization of the alcohol. The dried milled wheat germ was hydrolyzed by refluxing with 1.0 N methanolic NaOH at 80 °c for 45 minutes. The product hydrolyzed are consists of long chain alcohol (policosanol) and free fatty acid. The purification of hydrolyzed products was performed by extraction with dichloromethane twice follow by pH adjustment to 7 using 6 N hydrochloric acid thereby the purity of policosanol was confirmed by TLC. The aliphatic long chain alcohols composition in policosanol were establish in 18-34 carbon atoms analyzed by gas chromatographic technique (GC). Wheat germ can be a viable policosanol source for health benefits product development or food supplements.

***Keywords***: *Beeswax; Hydrolysis; Microwave; Policosanol*

## 1. Introduction

Policosanols (PC) are a mixture of high molecular weight of primary aliphatic long chain alcohol (20-36 carbon atoms) containing mainly docosanol, tetracosanol, hexacosanol, octacosanol and triacontanol [1]. The ratios between these substances tend to be different from policosanol supplements. Policosanol seems to reduce lipid levels and treat intermittent claudication through several biological mechanisms, including reduced platelet aggregation, increased antioxidant activity, increased bile acid secretion, inhibition of hepatic cholesterol synthesis [2-10]. For most people, policosanol is a natural product. Side-effects are few or non-existent, while clinical efficacy has proven to be comparable or even better. The policosanol is produced from sugar cane wax, beeswax, or wheat germ. [1,2].

The technique for policosanol extraction is hydrolysis or saponification reaction with NaOH or KOH base in various times and temperatures. The wax ester was hydrolyzed into long chain alcohols or policosanol and free fatty acids organic solvents phase such as n-hexane, toluene, benzene, ethyl acetate, ethanol, methanol and acetone in preparing the NaOH or KOH base for catalyst the reaction. [1,11-17]. Moreover, the researchers have investigated on the use of enzyme catalyst methanolysis in supercritical carbon dioxide [18] using High ultrasound (HIU) as catalyst [19] and using pressurised solvent extraction [2].

For policosanol extracted purification have to washing with organic solvents [1,2,5,11-16,20] and following by crystallization [21]. Thereby, the suitable solvent selection is the most important step in policosanol purification for optimizing recovery of aspiring to the main components from a complex mixture. The most commonly technique for policosanol analyzation and characterization can be done by TLC, GC and HPLC or combining these technique [5,14-15,22,23].

Policosanol was originally isolated from sugar cane wax and is also found in a number of other natural substances such as beeswax, rice bran, and wheat germ [23]. These long-chain primary alcohols are present in fruit, leaves and surfaces of plants, and whole seeds [1]

Wheat is among the most extensively grown crop in the world. Bioactive wheat components are concentrated in the outer layers of the grain [24], the surfaces usually have a layer that

contains wax. The isolation of octacosanol from wheat was first reported by Pollard in 1933 [25] Later, Tulloch examined the octacosanol composition in durum, club, and wheat leaf wax [26-27] and Several studies reported the presence of policosanol in wheat leaf wax [28-29]. The principal component of the wax from blades of young wheat was identified as a long-chain alcohol, octacosanol. Policosanol contents and compositions of wheat germ oil (WGO), straw and grain fractions have also been published [1,3,23-24]. According to these studies wheat can be a good source of policosanol. The examination of wheat such as straw, bran and germ as potential sources of policosanol will provide valuable information.

Although the policosanol composition of plant wax has been studied extensively, long chain alcohol compositions have been observed in various original of wax. In this study, Wheat is once of economic plants of Thailand, it can be growth in northern Thailand area to be used to raw material. The policosanol were extraction and purification from wheat germ by hydrolysis reaction. The efficiency of aliphatic long chain alcohols isolation and purification were done after hydrolyzation. Due to the ratios

of alcohol composition tend to be different from policosanol supplements. Therefore, the characterization of the alcohol was to establish through the use of GC.

## 2. Materials and Method

### A. Materials and reagents

Wheat germ and rice bran oil (as triglyceride) in food grade was purchased from local supermarket in Chiangmai, Thailand. The moisture content of wheat germ was evaluate after at 105 °c by hot- air oven and all the results were determined in a dry matter basis. Chemical reagents in analytical grade were provided from LabScan (Bangkok, Thailand). Standard hydrocarbons and aliphatic long chain alcohols were supplied by Sigma Aldrich, Thailand.

### B. Policosanol extraction

The dried milled wheat germ in 10 grams was hydrolyzed with 100 ml of 1.0 N methanolic NaOH at 80-85 °c for 45 minutes by refluxing with continuous stirring. After cooling, the hydrolyzed wheat germ was filtrated into two fraction which are supernatant methanol solution fraction and hydrolyzed wheat germ precipitate fraction. The methanol fraction was evaporated to dryness. The dry residues were checked via TLC analysis for product of hydrolyzation testing.

### C. Policosanol purification

The hydrolyzed wheat germ was purified by washing with 50 ml of dichloromethane. The mixture was shaken and stored at room temperature for 24 hr. using a separation funnel. The extraction was repeated 3 times using equal volumes of dichloromethane. The extraction had separated into dichloromethane solution phase and mud cake phase. The dichloromethane collected from three extractions was combined and prepared for TLC test. The mud cake collected was added with 1N HCl until the pH of the aqueous phase reached 7 before TLC analysis.

### D. Thin layer chromatography

The hydrolyzed product in the part of methanol fraction and the purified policosanol (dichloromethane solution phase and mud cake phase) were analyzed on preparative silica gel thin-layer plates using one-dimensional TLC with chloroform: hexane: acetic acid (70: 30: 1, v/v/v) as the developing solvent. The samples were applied on the silica gel plates to correctly identify with the standard aliphatic alcohol bands, a reference. After development the plate was applied into iodine tank for analysis.

### E. High performance chromatography

The quantification of FFA were studied by HPLC via free fatty acid as external standard. Moreover, the quantification of FA were studied by HPLC via free fatty acid as external standard. The FFA analysis was carried out by HPLC using acetronitrile/methanol (4:1) solvent system as a mobile phase and C18 (Hewlett Packard) HPLC column (125 mm x 4.0 mm i.d.).

### F. Analysis of policosanol composition

Policosanol composition from wheat germ extraction was analyzed by capillary column of GC-FID. The column was HP-5 capillary (5% phenyl-95% diethylpolysiloxane (30 m x 0.25 mm, 0.25 µm film thickness). Oven temperature was as follows: 3 min at 150 °C; from 150 to 280 °C with 15 °C /min heating rate and maintained at this temperature for 10 min. Duplicate samples (1 ml) were injected into GC with split less. Policosanol compositions of the samples were determined by comparison of their chromatographic retention times with those of the standards and confirmed with long chain alcohols standards octadecanol (C18-OH), eicosanol (C20-OH), docosanol (C22-OH), tetracosanol (C24-OH), hexacosanol (C26-OH), octacosanol (C28-OH), triacontanol (C30-OH) and dotriacontanol (C32-OH).

## 3. Results and Discussion

### G. Moisture content of wheat germ

Physical properties of crude wheat germ is a moisture content as an important factor for the extraction. The moisture content of wheat germ of northern Thailand shows in 5.4686 ± 0.1606 % (w/w) which is near to the data of Moran, 1967 [30]. The data of moisture were summarized in table I.

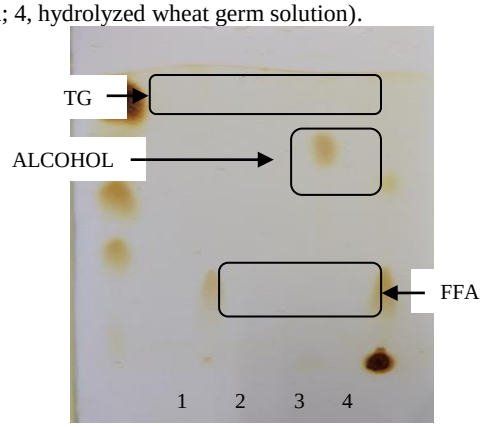
**Table I** percentage of moisture content in wheatgerm

| Treatment | Weight (g) | | Moisture (% w/w) |
|---|---|---|---|
| | Wet (g) | Dry (g) | |
| 1 | 2.6576 | 2.5139 | 5.4071 |
| 2 | 2.6646 | 2.5133 | 5.6887 |
| 3 | 2.6573 | 2.5162 | 5.3099 |
| **Average** | 2.6599 | 2.5145 | **54686± 0.1606** |

### H. Policosanol extraction by hydrolysis

The extraction of dried milled wheat germ by base catalyzed hydrolysis can be done by refluxing with 1 N methanolic NaOH at 80-85 °C for 45 minutes. The concentration of NaOH had effect to soluble efficiency in polar and non-polar organic solvents due to the simultaneous solution of the reaction is very importance. Moreover, the advantage of NaOH solution in methanol is to protection the possible emulsion formation of the reaction. The NaOH solution be prepared in methanol to use as catalyst for wheat germ hydrolysis in policosanol extraction. The TLC in Figure 1 had been present to complete hydrolysis of the wheat germ. Because bioactive wheat components are concentrated in the outer layers of the grain, the surfaces usually have a layer that contains wax. Thereby the wax ester did not appear on the TLC plate due to the fact that hydrolyzation of the wax to long chain aliphatic alcohols and free fatty acids. In addition, wheat germ contains large amount of triacylglycerol (TAG), about 10–12% (w/w) [31] so, it had been complete hydrolyzed to the free fatty acid to show in the TLC plate. The results had corresponded with the researchers who had used NaOH base prepared in organic solvent for catalyst the hydrolysis reaction of wheat germ using refluxing technique [1-3,11].

Fig. 1. Thin layer chromatography of hydrolyzed wheat germ (1, rice bran oil (TG); 2, std. free fatty acid (FFA); 3, std. stearyl alcohol; 4, hydrolyzed wheat germ solution).

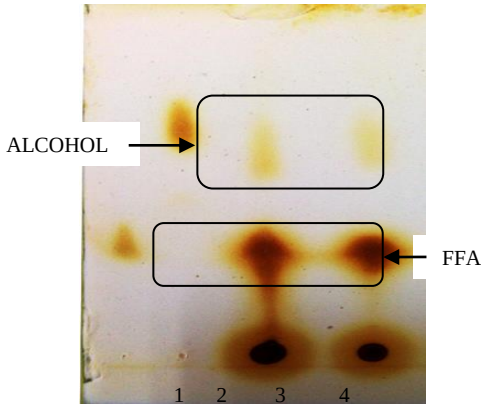



### I. Policosanol purification and composition identification

The hydrolyzed wheat germ are compost of long chain aliphatic alcohols and free fatty acids. The aim of hydrolyzed wheat germ purification is to elimination of free fatty acids. It was done by washing with dichloromethane and following by pH adjustment. The results of first step of washing with dichloromethane and stored at room temperature had showed into dichloromethane solution phase and mud cake phase. The result of purified hydrolyzed wheat germ was tested by TLC showing in Figure 2. In spot of dichloromethane solution phase exhibit in two spots of long chain aliphatic alcohols and free fatty acids as same

as non-purify extracted. The dichloromethane solvent using in the first step of purification cannot use to purify the policosanol due to the sodium salt of free fatty acid can be dissolved in dichloromethane too.

Fig. 2. Thin layer chromatography of purified policosanol (1, std. free fatty acid (FFA); 2, std. stearyl alcohol; 3, hydrolyzed wheat germ solution,; 4, dichloromethane fraction).




While the mud cake phase was separating from dichloromethane solution phase, it was pH adjustment to pH 7 then, the purity of policosanol was qualified by TLC. After the pH adjustment of mud cake, it divided into solvent layer (dichloromethane) on the top and aqueous layer in the bottom. The comparison of pH adjustment effect on purification was displayed on TLC plate in Figure 3. The result of policosanol purity via reducing of free fatty acid was confirmed by HPLC displaying in Figure 4. The policosanol exhibit in high purity in dichloromethane solvent layer whereas the free fatty acid impurity increasing was found in aqueous layer. The result show capable of efficiently extracting policosanol thereby the residual sodium salt of fatty acid can be reduced by pH adjustment.

Fig. 3. Thin layer chromatography of high purity policosanol via pH adjustment (1, std. free fatty acid (FFA); 2, std. stearyl alcohol; 3, hydrolyzed wheat germ solution; 4, dichloromethane fraction (non pH adjusted); 5, dichloromethane fraction (pH adjusted); 6, aqueous fraction (pH adjusted)).

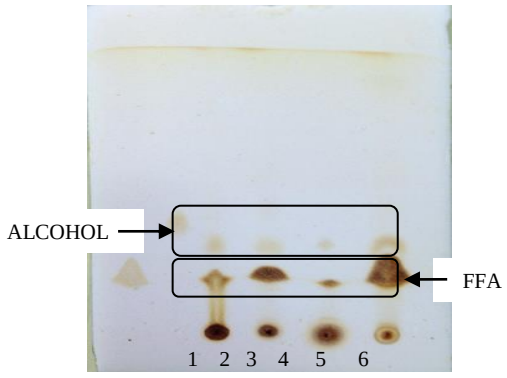
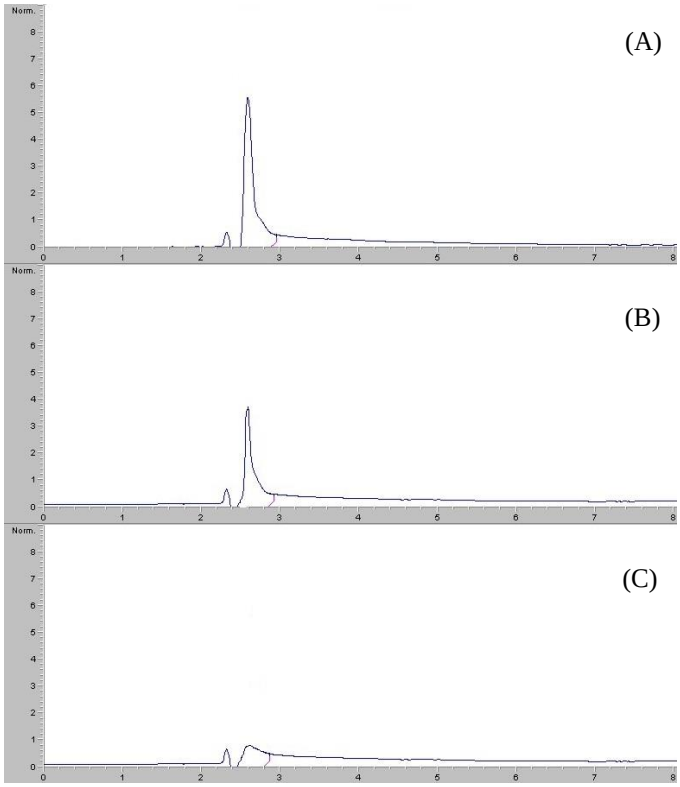



Fig. 4. Chromatography of policosanol purification (A; std. free fatty acid (FFA); B, dichloromethane fraction (non pH adjusted); C, dichloromethane fraction (pH adjusted).




The analysis of individual policosanol components required GC for identification of the long chain alcohols type. The chromatogram of GC-FID shows peak of standard aliphatic long chain alcohol (Figure 5 (A)) and long chain alcohol or policosanol components separated from wheat germ extracted which purified with dichloromethane followed by pH adjustment, their identities are reported in Figure 5 (B). The policosanols compositions were identified by direct comparison of their chromatographic retention times with those of authentic compounds in Figure 5. The aliphatic molecules consist of long chain alcohol length ranging from C18-OH to C32-OH in following various ratio: C18-OH, 28.52%; C20-OH, 54.96%; C22-OH, 10.45%; C24-OH, 0.23%; C30-OH, 1.98%; C32-OH, 3.86%. The data of and percentage of long chain alcohols were summarized in table II. The main constituents of the aliphatic alcohol fractions are eicosanol (C20-OH), accounting for over 50% of the total aliphatic alcohol content ratio. Octadecanol (C18-OH) and docosanol (C22-OH) were in range of 10-30%. Whereas tetracosanol (C24-OH), triacontanol (C30-OH) and Do- triacontanol (C32-OH) amounted to less than 5% of the total aliphatic alcohol amount. However, Lin (2004) had reported the long chain alcohol composition of wheat germ showing in range C24-OH to C34-OH in various ratio. Therefore, the variation of long chain aliphatic alcohol type and content in policosanol depend on the origins of wheat germ varieties and growth area such as Asia, America, European or African zone.

Fig. 5. Chromatography of aliphatic long chain alcohol composition (A; std. long chain alcohol; B, extracted from wheat germ).

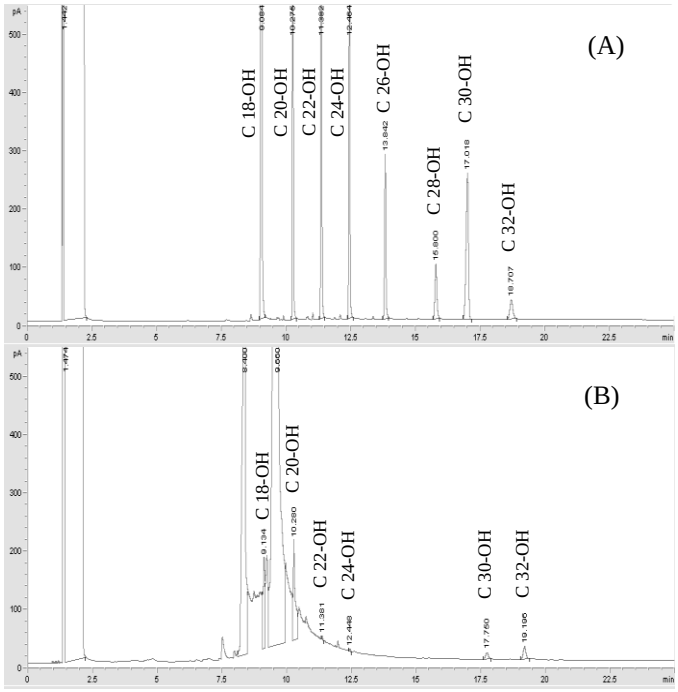
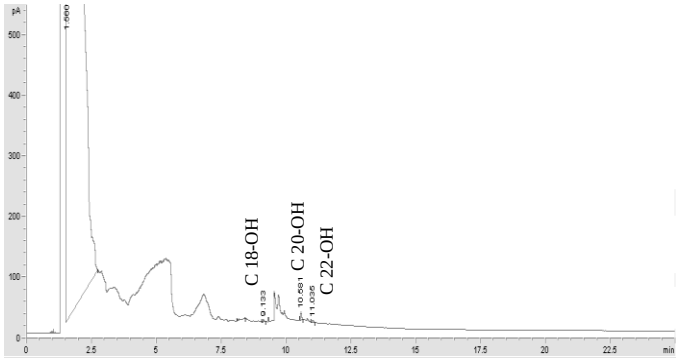



**Table II** percentage ratio of long chain alcohols composition in wheatgerm

| Alcohol type | % Ratio | |
|---|---|---|
| | Experimental | Lin (2004) [23] |
| Octadecanol (C18-OH) | 28.52 | - |
| Eicosanol (C20-OH) | 54.96 | - |
| Docosanol (C22-OH) | 10.45 | - |
| Tetracosanol (C24-OH) | 0.23 | 2.1 |
| Hexacosanol (C26-OH) | - | 8.4 |
| Octacosanol (C28-OH) | - | 67.9 |
| Triacontanol (C30-OH) | 1.98 | 12.6 |
| Dotriacontanol l (C32-OH) | 3.86 | 5.9 |
| Tetratriacontanol (C34-OH) | - | 3.1 |
| **Total** | 100 | 100 |

Thereby, in the pH adjustment purification step it divided into dichloromethane solvent layer and aqueous layer. The result of policosanol purity as in Figure 3 had showed in free fatty acid impurity increasing in the aqueous layer. The dichloromethane layer spot on the TLC in Figure 3 was found long chain alcohol so that it was to identifing the composition of the long chain alcohol. The aliphatic molecules of long chain alcohol consists of C18-OH, C20-OH and C22-OH. The profiles of the alcohol had showed in Figure 6 and it was analyzed by direct comparison their chromatographic retention times with the standard aliphatic long chain alcohol.

Fig. 6. Chromatography of aliphatic long chain alcohol composition in aqueous layer from pH adjustment.




# 4. Conclusion

Wheat germ from northern Thailand show high efficiency to their respective aliphatic long chain alcohols and long chain fatty acid by the hydrolysis reaction. The aliphatic long chain alcohols composition in policosanol showed in octadecanol (C18-OH), eicosanol (C20-OH), docasanol (C22-OH), tetracasanol (C24-OH) and triacontanol (C30-OH). The high purity and long chain alcohols component of policoanol extracted from wheat germ have many potentials in applications.

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