The Policosanol Extraction and Composition Characterization from Wheat Straw By-Product of Thai Wheat Varieties

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***Abstract*—Policosanol is a group of long chain primary aliphatic alcohols (20-36 carbon), which are constituents of plant epicuticular waxes. Policosanol enriched dietary supplements and functional foods are commercially available in the world market for their low density lipoprotein lowering properties. The presence of policosanol in the surfaces layer of wheat straw has been reported previously. The of this study was to examine the Policosanol extraction technique and composition from wheat straw by-product of Thai wheat varieties. This study show that the suitable solvent selection is the most important step in policosanol extraction and purification for optimizing recovery of aspiring to the main components from a complex mixture. Policosanol from wheat straw contain octadecanol, eicosanol, tetracosanol, hexacosanol and octacosanol. Specifically, wheat straw exhibit highest octacosanol content ratio in MJU 3, MJU 2 and Fahng 60 varieties, respectively. This study demonstrated that wheat varieties grown under identical growing conditions and management have differ significant effects on policosanol composition.**

***Index Terms*—hydrolyzation, policosanol, solvent extraction, wheat straw**

I. Introduction

Wheat is among the most extensively grown crop in the world. Bioactive wheat components are concentrated in the outer layers of the grain [1]. Wheat grain is only 40% of the biomass produced by the crop. wheat straw is an abundant byproduct from wheat production. One hectare of wheat produces more than 4.8 tons of straw [2]. The average yield of WS is 1.3–1.4 kg/kg of wheat grain [3]. At present only about 3.2% of the economic return on wheat is from straw [4].

Currently, straw is baled for use as livestock bedding or low-grade animal feed providing minimal return. To be converted to ethanol and other value-added products.

Wheat straw is a lignocellulosic material containing approximately 35–40% cellulose, 30–35% hemicellulose, and 10–15% lignin [5].

Much of the research on pretreatment of straw and lignocellulose in general has been focused on conversion of the cellulose to fermentable glucose using cellulase enzymes, which are used to convert the solid cellulose to fermentable glucose. Further research is needed to improve the feasibility of converting straw into bio-ethanol or/ and other value-added products.

Currently, a number of dietary supplements containing policosanols are commercially available in the world market. Policosanols are a group of long chain aliphatic alcohols, with chain lengths varying between 20 to 36 carbon atoms, specifically octacosanol (C28-OH). Octacosanol is reported to be the bioactive policosanol component in various dietary supplements [6]-[8]. Policosanol is one of component having the potential to be used in food application and pharmaceutical.

Policosanols will be recommended as part of the strategy to lower serum lipids. A number of animal and clinical studies have demonstrated that policosanol consumption had effect on intermittent claudication, reducing plated aggregation and LDL peroxidation and endothelial function and aiding in the prevention of cardiovascular disease [6], [9]-[14]. Policosanols has also beneficial effects on smooth muscle cell proliferation [6], [9], [10]-[12].

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 [15]. Compositions, novel isolation sources and processes, and uses for unique policosanol containing compositions are disclosed. These long-chain primary alcohols are present in fruit, leaves and surfaces of plants, and whole seeds [16].

Policosanol contents and compositions of wheat germ oil, straw and grain fractions have also been published [9], [16]. The examination of wheat such as straw, bran and germ as potential sources of policosanol will provide valuable information.

The policosanols are isolated and may be combined with other phytochemical/processing products, and are used in numerous food, beverage, health and/or nutraceutical applications. Seed and vegetable processing byproduct and/or "waste" streams are disclosed as containing policosanols with various relative amounts of the various long chain fatty/waxy alcohols. The objective of this study was to examine the policosanol extraction

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technique from wheat straw by-product of Thai varieties and effect of solvent type for policosanol purification and characterization of the alcohol by GC for providing valuable information.

II. Materials and Methods

A. Materials

Wheat straw were obtained from wheat varieties grown at Maejo University in Chiang Mai campus, Chiang Mai Thailand (MJU 2, MJU 3, MJU 6 and Fahng 60). Wheat straw samples has been dried (at 60 ℃ by hot- air oven, 6 hr.) and milled before stored in the same conditions. Standard aliphatic long chain alcohols were supplied by Sigma Aldrich, Thailand. Chemical reagents in analytical grade were provided from LabScan (Bangkok, Thailand). Acetronitrile and Methanol in HPLC grade were purchase from Fisher Chemicals (UK).

B. Hrdrolyzation Procedure for Policosanol Extraction

The wheat straw samples were analyzed the moisture by content hot-air oven at 105 ℃ and all the results were determined in a dry matter basis. The dried sample were milled in a blender for 10 minutes. The dried milled wheat straw sample was hydrolyzed by refluxing with 1.0 M NaOH in ethanol for 2 hours at 80-85 ℃. The hydrolyzed sample was cooled and filtered through filter paper. The hydrolyzed sample was tasted by TLC for hydrolyzation completion check with chloroform: hexane: acetic acid (70: 30: 1, v/v/v) as developing solvents.

C. Extraction Procedure for Policosanol Purification

The hydrolyzed sample was washed with 10% HCl until neutrality and the extraction sample were performed using four different solvents (heptane, ethyl acetate, petroleum ether, acetone) at 85-90 ℃ using 100 mL volume extraction cells and followed by washing with ethanol. Then, the extractions were extracted three times using equal volumes of diethyl ether of the samples and following washing with hot water until the pH of the water phase became neutral. The solvent phase was collected to quantification study of free fatty acid (FFA) impurities for purification checking of policosanol extracted by HPLC via FFA as external standard. The 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.)

D. Composition Identification of Policosanols

The purified policosanol was determined policosanol composition 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 ℃; from 150 to 280 ℃ with 15 ℃ /min heating rate and maintained at this temperature for 10 min. The samples (1 μL) were injected into the GC by an autosampler and spitless. The policosanol compositions of the samples were identified by direct comparison of their chromatographic retention times with those of the standard compounds. The individual policosanol standards used for peak identification and quantification, 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).

III. Result and Disscussion

A. Hrdrolyzation for Policosanol Extraction

The long chain alcohol extraction from dried milled wheat straw can be done by hydrolyzation with 1 M ethanolic NaOH at 80-85 °C for 2 hrs. Wheat straw is a lignocellulosic material containing such as cellulose, hemicellulose and lignin. The strong base NaOH in ethanol show high efficiency in hydrolyzation due to it had effect to simultaneous soluble in polar and non-polar substance.

The TLC plate had been present to complete hydrolyzation of the sample (MJU 2, MJU 3, MJU 6 and Fahng 60) as shown in Fig. 1. Because bioactive in the surfaces of wheat straw have a layer that contains wax [17]. 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.

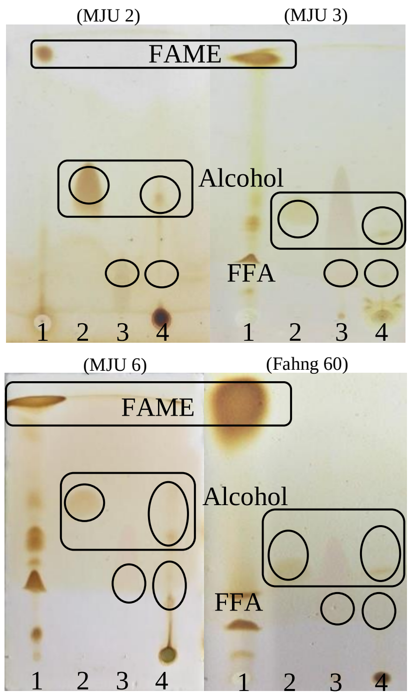

Figure 1. Thin layer chromatography of complete hydrolyzation wheat straw (1, fatty acid methyl ester of rice bran oil (FAME); 2, std. stearyl alcohol; 3,std. free fatty acid (FFA); 4, hydrolyzed wheat straw solution).

B. Extraction for Policosanol Purification

The hydrolyzed wheat straw show in long chain aliphatic alcohols and free fatty acids of the wax ester from the surfaces layer of the raw wheat straw. The free fatty acids must be eliminated from the long chain alcohols to purify the hydrolyzed wheat straw. Firstly, the hydrolyzed sample was washed with 10% HCl until neutrality after that the difference properties of solvent were used in purification. The dielectric constant identifies the polarity of a solvent and a key parameter to determine solute–solvent interactions. It involves dispersion dipolar and/or multi-polar interactions. Solvents with a wide range of dielectric constants, hepane (1.9), petroleum ether (4.3), ethyl acetate (6) and acetone (20.7) and the hydrolyzation of wheat straw from Fahng 60 wheat varieties were chosen for this study.

The solvent phase was collected to quantification study of free fatty acid (FFA) impurities. The HPLC in Fig. 2 had been present to qualification of the free fatty acids thereby being carried out using of stearic acid as an external standard (Fig. 2 (A)). The effect of solvent type on the amount of FFA extract was significant only when wheat straw extracted with acetone which had the highest dielectric constant. It is expected that sodium salt of long chain fatty acid can be dissolved in acetone too.

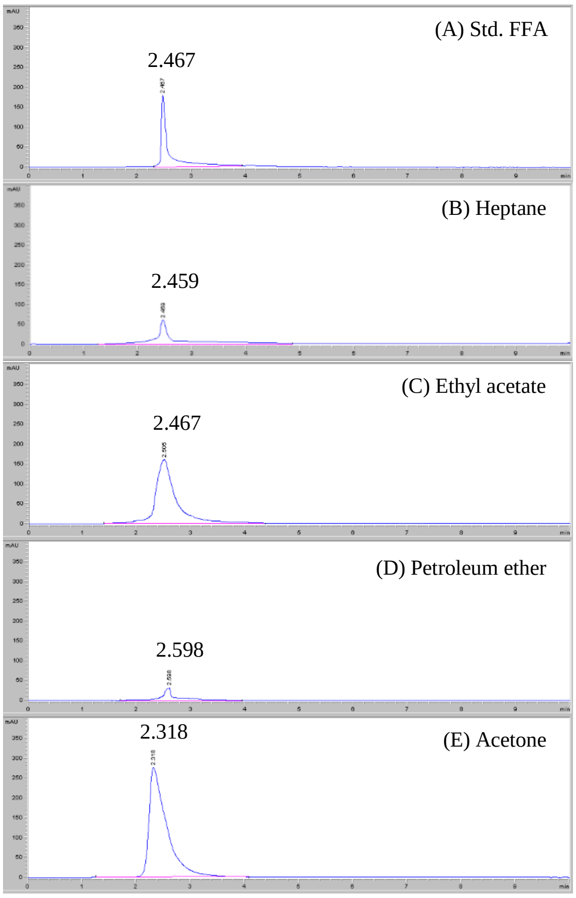

Figure 2. Chromatograms of free fatty acid (FFA) impurities extracted by organic solvent extraction of hydrolyzed Fahng 60 wheat varieties (A) Std. FFA, (B) heptane extracted, (C) ethyl acetate extracted, (D) Petroleum ether extracted, (E) acetone extracted.

However, the policosanol purity was washed again with ethanol which can be reducing the simultaneous wheat straw hydrolyzed components, thereby clear of the layer separation. Then, the solvent layer was repeated extraction for purification increasing in three times with diethyl ether. 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. Moreover, the residual sodium salt of long chain fatty acid can be eliminated by washing with hot water until pH of the aqueous phase reached 7. The result in Fig. 3 show capable of efficiently increasing policosanol purity extracted from the sample wheat straw of wheat varieties (MJU 2, MJU 3, MJU 6 and Fahng 60). Thereby the free fatty acid can be removed by extracted with ethanol, the low dielectric constant solvents (diethyl ether) and washing with hot water.

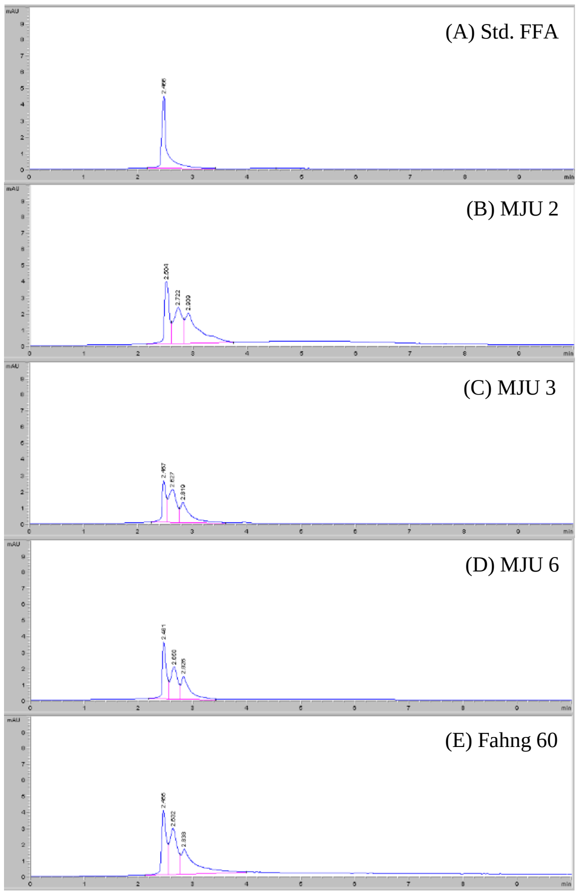

Figure 3. Chromatograms of free fatty acid (FFA) extracted by diethyl ether purification of wheat varieties (A) Std. FFA, (B) MJU 2 varieties, (C) MJU 3 varieties, (D) MJU 6 varieties, (E) Fahng 60 varieties.

C. *The Composition of Policosanol*

Policosanol compositions of the samples were determined by comparison of their chromatographic retention times in chromatogram of GC-FID with those of the authentic standards compounds in Fig. 4. The chromatogram of GC-FID shows peak of standard aliphatic long chain alcohol (Fig. 4 (A)) and long chain alcohol or policosanol components separated from wheat straw extracted from MJU 2, MJU 3, MJU 6 and Fahng 60 wheat varieties grown in Chiang Mai Thailand (Fig. 4 (B)-(E)).

Octadecanol (C18-OH), eicosanol (C20-OH), tetracosanol (C24-OH), hexacosanol (C26-OH), octacosanol (C28-OH) were detected in wheat straw extracted sample. The data of type and percentage of long chain alcohols were summarized in Table I. Similar policosanol composition data for wheat straw were publisher by [9], [18].

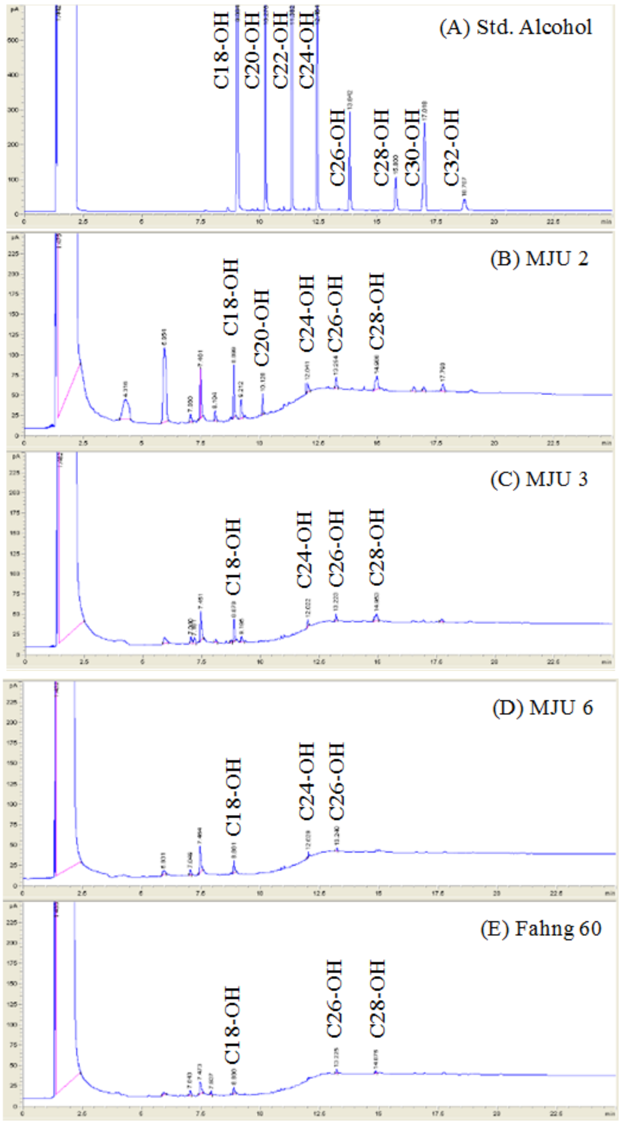

Figure 4. Chromatograms show aliphatic long chain alcohol composition of wheat varieties (A) Std. long chain alcohol, (B) MJU 2 varieties, (C) MJU 3 varieties, (D) MJU 6 varieties, (E) Fahng 60 varieties.

TABLE I. LONG CHAIN ALCOHOL COMPOSITION OF WHEAT STRAW VARIETIES

Long Chain Alcohol	Alcohol Ratio (%)			
	MJU 2	MJU 3	MJU 6	Fahng 60
Octadecanol (C18-OH)	41.53	39.92	62.42	53.70
Eicosanol (C20-OH)	11.62	-	-	-
Tetracosanol (C24-OH)	9.31	11.08	21.46	-
Hexacosanol (C26-OH)	10.04	13.27	16.12	28.40
Octacosanol (C28-OH)	27.50	35.73	-	17.90

Octacosanol (C28-OH), especially, contents of wheat straw samples varied between 18-35% of the total alcohol content ratio. MJU 2 and MJU 3 had significantly higher octacosanol content than Fahng 60. Wheat straw exhibited significantly high octacosanol (C28-OH) content. Octacosanol is reported to be the bioactive policosanol component in various dietary supplements carried out with tail-suspended rats indicated that octacosanol could counteract some effects of simulated weightlessness on rats [6]-[8], suggesting that octacosanol enriched foods might benefit astronauts during space travels. Therefore, the high octacosanol content of MJU 2 and MJU 3 and Fahng 60 wheat varieties also make these varieties a candidate as a source of policosanol for functional foods and nutraceutical applications.

IV. CONCLUSIONS

Wheat straw can be a viable policosanol source for commercial application. It was found that significant variations existed in policosanol composition among wheat varieties. Organic solvent extraction of wheat straw was effective in policosanol purity. The MJU 2 and MJU 3 and Fahng 60 varieties also contained a very high amount of octacosanol (C28-OH), indicating that these varieties may be potential policosanol sources for functional foods, dietary supplements and pharmaceutical applications. However, policosanol recovery process from wheat straw must be required research data for increasing of wheat straw by-product economic valuation.

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REFERENCES

[1] Y. Lin, R. Mike, P. J. W. Reggy, A. T. Elke, M. Gerald, S. Aafje, and W. M. Gert, “Wheat germ policosanol failed to lower plasma cholesterol in subjects with normal to mildly elevated cholesterol concentrations,” *Metabolism*, vol. 53, pp. 1309-1314, Oct 2004.

[2] B.C. Saha, L. B. Iten, M. A. Cotta, and Y. V. Wu, “Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol,” *Process Biochemistry*, vol. 40, pp. 3693–3700, Apr 2005.

[3] D. Montane, X. Farriol, J. Salvado, P. Jollez, and E. Chernet, “Application of steam explosion to the fractionation and rapid vapour-phase alkaline pulping of wheat straw,” *Biomass Bioenergy*, vol. 14, pp. 261–276, Mar 1998.

[4] R. L. Hoskinson, J. R. Hess, T. D. Foust, W. T. McKean, and M. S. Lewis, “Fractionation of higher value crop residue components for conversion into bioenergy and industrial products,” presented at the ASAE Annual International Meeting, Sacramento, California, USA, July 29-August 1, 2001.

[5] S. H. Harper and J. M. Lynch, “The chemical components and decomposition of wheat straw leaves, internodes and nodes,” *Journal of the Science of Food and Agriculture*, vol. 32, pp. 1057–1062, Nov. 1981.

[6] I. Gouni-Berthold and H. K. Berthold, “Policosanol: Clinical pharmacology and therapeutic significance of a new lipid-lowering agent,” *American Heart Journal*, vol. 143, pp. 356-365, Feb. 2002.

[7] J. C. Taylor, L. Rapport, and G. B. Lockwood, “Octacosanol in human health,” *Nutrition*, vol. 19, pp. 192-195, Feb. 2003.

[8] S. Bai, L. Xie, C. Liu, Q. Zheng, and J. Chen, “Effects of octacosanol in food physiological parameters in tail-suspended rats,” *Space Medicine & Medical Engineering*, vol. 10, pp. 450-452, Dec. 1997.

[9] S. Irmak and N. T. Dunford, “Policosanol contents and compositions of wheat varieties,” *Journal of Agricultural and Food Chemistry*, vol. 53, pp. 5583-5586, Jul. 2005.

[10] V. Fraga, R. Menendez, A. M. Amor, R. M. Gonzalez, S. Jimenez, and R. Mas, “Effect of policosanol on in vitro and in vivo rat liver

microsomal lipid peroxidation," *Archives of Medical Research*, vol.28, pp. 355-360, 1997.
[11] S. Kato, K. I. Karino, S. Hasegawa, J. Nagasawa, A, Nagasaki, *et al*., "Octacosanol affects lipid metabolism in rats fed on a high-fat diet," *British Journal of Nutrition (London)*, vol. 73, pp. 433-441, Mar. 1995.
[12] S. Valdes, M. L. Arruzazabala, and E. Fernandez, "Effect of policosanol on platelet aggregation in healthy volunteers," *International Journal of Clinical Pharmacology Research*, vol. 16, pp. 67-72, Feb. 1996.
[13] F. Francini-Pesenti, F. Brocadello, D. Beltramolli, M. Nardi, and L. Caregaro, "Sugar cane policosanol failed to lower plasma cholesterol in primitive, diet-resistant hypercholesterolaemia: A double blind, controlled study," *Complementary Therapies in Medicine*, vol. 16, pp. 61-65, Apr. 2008.
[14] S. Harrabi, S. Boukhchina, P. M. Mayer, and H. Kallel, "Policosanol distribution and accumulation in developing corn kernels," *Food Chemistry*, vol. 115, pp. 918-923, Aug. 2009.
[15] B. Andrea and H. Alyssa, "Wheat germ: Not only a by-product," *International Journal of Food Sciences & Nutrition*, vol. 63, pp. 71–74, Mar. 2012.
[16] S. Irmak, N. T. Dunford, and J. Milligan, "Policosanol contents of beeswax, sugar cane and wheat extracts," *Food Chemistry*, vol. 95, pp. 312-318, Mar. 2006.
[17] F. E. I. Deswarte, J. H. Clark, J. J. E. Hardy, and P. M. Rose, "The fractionation of valuable wax products from wheat straw using CO_2," *Green Chemistry*, vol. 8, pp. 39-42, Jan. 2006.
[18] N. T. Dunford, and J. Edwards, "Nutritional bioactive components of wheat straw as affected by genotype and environment," *Bioresource Technology*, vol. 101, pp. 422–425, Jan. 2010.

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