Wax: Co-Product of Rice Bran Oil Refining

Assoc. Prof. Dr. Kornkanok Aryusuk

Lipid Technology Laboratory

Biochemical Technology Division,

School of Bioresources and Technology,

KMUTT., Bangkok, Thailand

E-mail: kornkanok.ary@kmutt.ac.th

Outline

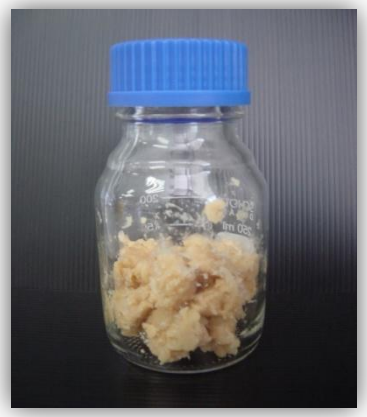
- Rice bran wax
  - Crude rice bran wax
  - Application of (pure) rice bran wax
  - Preparation of pure rice bran wax by transesterification process
- Policosanol
  - Application
  - Preparation
    - Saponification process
    - Transesterification process

Chemical Refining

Dewaxing

Neutralization

Bleaching



Dewaxing

Deodorization

(neutral oil, mainly (>99%) triglyceride)

Crude Rice Bran Wax (CRBW)

- **WAX ESTER (20-80%)**
- **Glycerides (20-80%)**
- **Free fatty acid**
- **Others**

Wax Esters

C_{22} and C_{24} C_{24} to C_{40}

Vali *et al*, 2005, “A process for the preparation of food-grad rice bran wax and the determination of its composition”, *JAOCS,* 82: 57-64.

Food and Drug Administration (FDA)

§ 172.890 Rice bran wax

Rice bran wax may be safely used in food in accordance with the following conditions:

(a) It is the **refined wax** obtained from rice bran and meets the following specifications:

- Melting point 75 – 80°C.
- Free fatty acids, 10% (max).
- Iodine number, 20 (max).
- Saponification number 75 – 120.

Food and Drug Administration (FDA)

§ 172.890 Rice bran wax

(b) It is used or intended for use as follows:

Food	Limitation in food	Use
Candy	50 ppm	Coating
Fresh fruits and vegetables	Do	Do
Chewing gum	2 1⁄2 pct	Plasticizing material

Others application of Rice bran wax

- Replace expensive carnuba wax for industrial application such as
  - cosmetics
  - medical applications
  - polishing wax
  - Rich source of **POLICOSANOL**

Preparation of Pure Rice Bran

You may notice in the former slide that

- FDA mentions only the refined wax from rice bran
- Applications of rice wax are in the refined (pure) form and other impurities must be removed.

Purification of RBW

Gopala Krishna et al, 1998

"A process for the preparation of >95% purified wax from wax sludge bran oil"

CRBW

Hydraulic pressing (5-50 psi)

Deoiled RBW

Non-polar solvent (1:2 ratio, stirring, RT, 30 min)

Refined RBW

Purification of RBW

Vali et al, 2005

A process for the preparation of food-grad rice bran wax and the determination of its composition, *JAOCS,* 82: 57-64.

CRBW

↓ Soxhlet extraction, 65^{o}C, 30 min, hexane

↓ Soxhlet extraction, 80^{o}C, 30 min, ISP

Defatted RBW

↓ Refluxed , 80^{o}C, 30 min ($NaBH_4$ in ISP)

↓ Cooled to RT, filtration

Refined RBW

(Mp 81-83^{o}C)

Purification of RBW

Gunawan et al, 2006

Purification and identification of rice bran oil fatty acid steryl and wax esters, *JAOCS*, 83: 449-456.

CRBW

Extraction (hexane)

Defatted RBW

Recrystallization in acetone (low T.)

Refined RBW

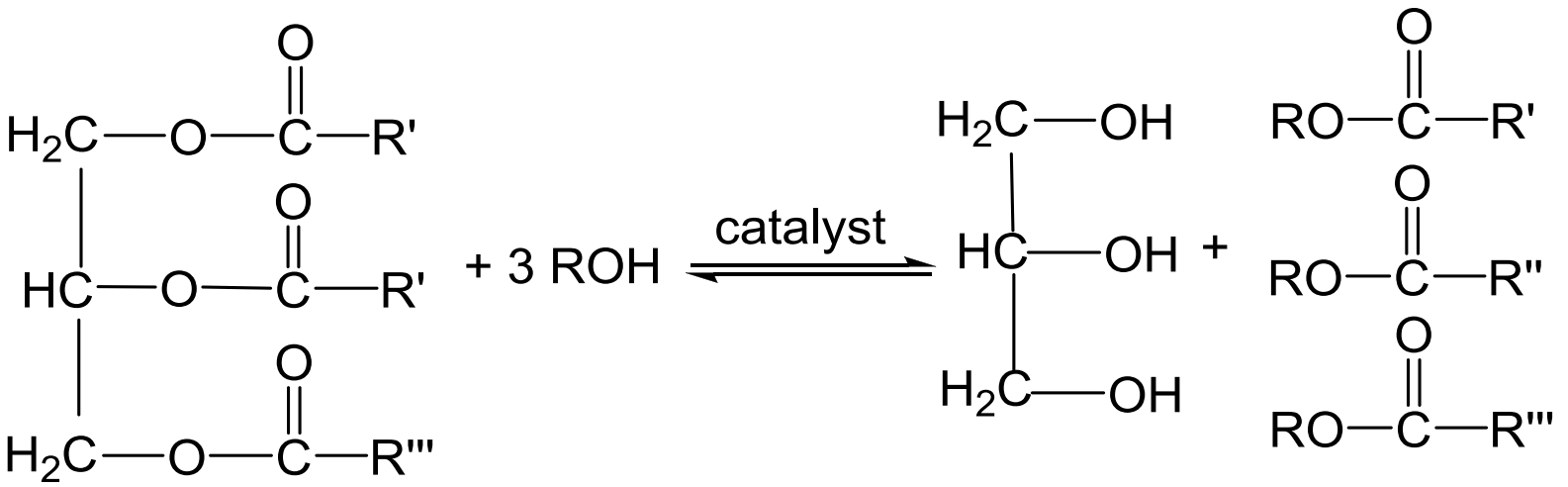
The Present Study

1. To prepare pure RBW (PRBW) by removing the glycerides from CRBW with the aim for:
   - Lower operation temperature & time
   - Reduce solvent uses
   - Use environmental-friendly solvent
2. Separation technique should be simplified

"Transesterification"

"Selective transesterification"

Transesterification



Triglyceride **3 Alcohol** **Glycerol** **3 Esters**

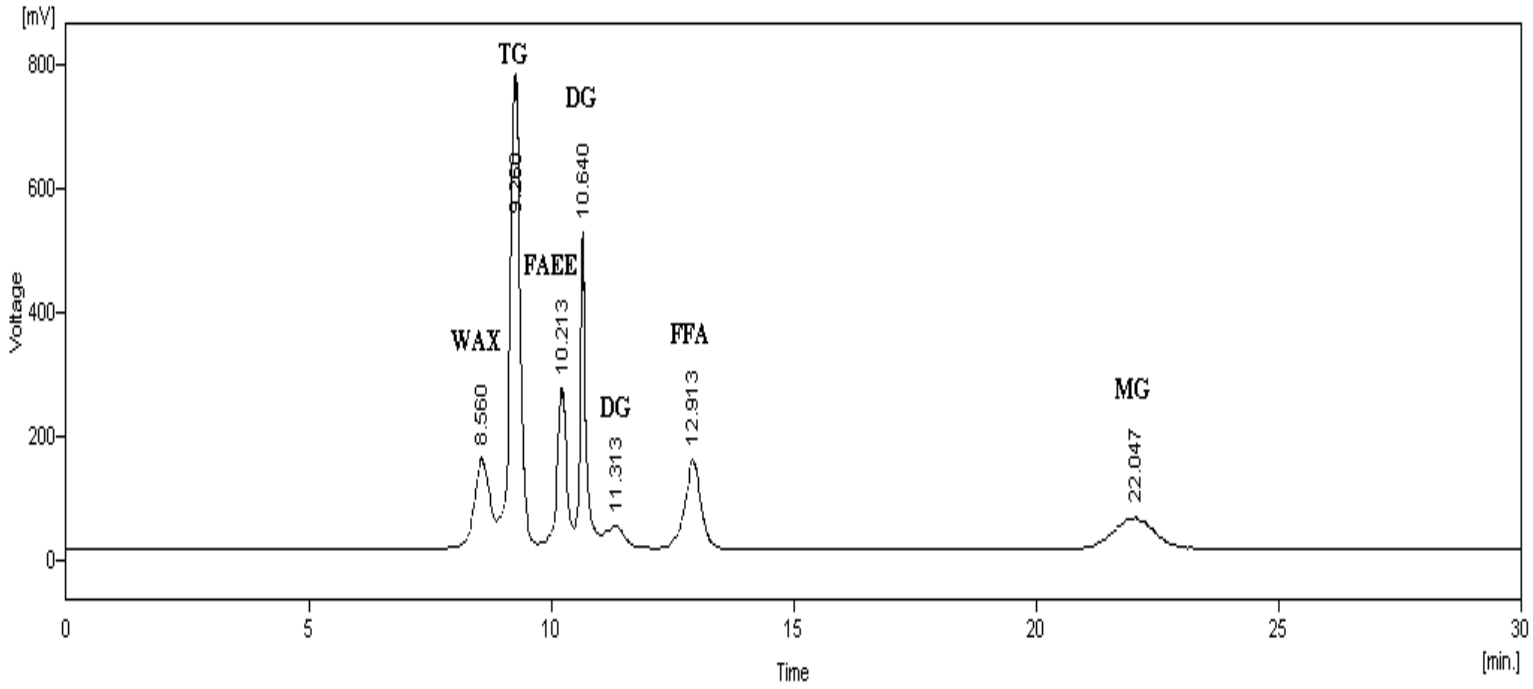


Fig. 1 HPSEC chromatogram of lipids on a 100-Å Phenogel column using isooctane/ toluene/ acetic acid (65:35:0.15, v/v/v) as mobile phase. Detector: ELSD, column & injector temp: 60ºC

Aryusuk, et.al, 2011, “Separation and determination of wax content using 100-Å Phenogel column”, *JAOCS*, 88: 1497-1501.

CRBW

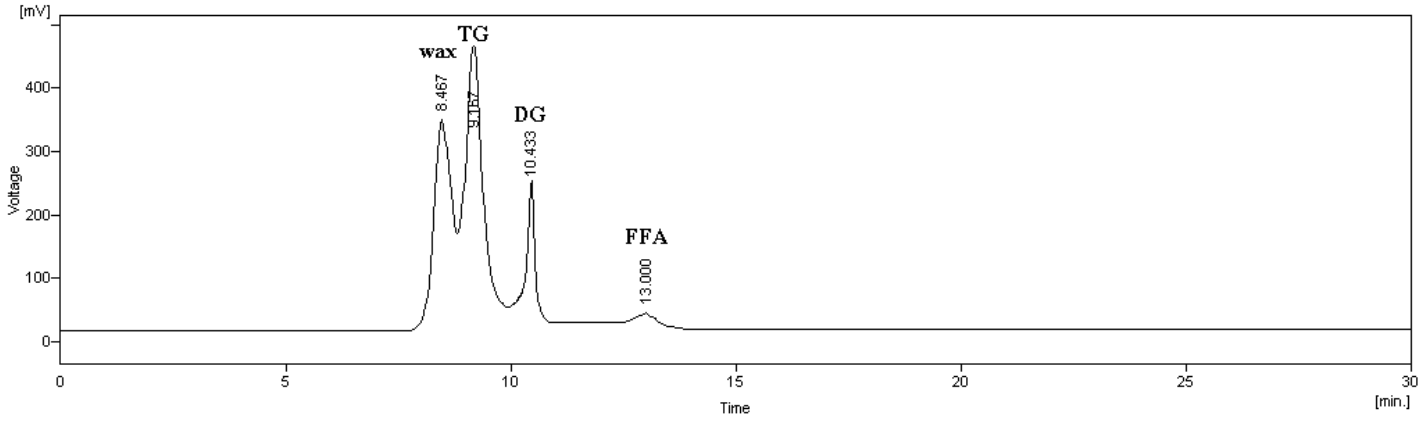
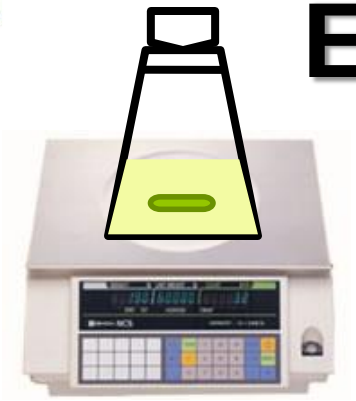


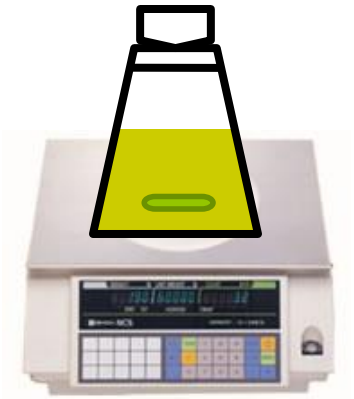
Fig. 2 HPSEC chromatogram of CRBW on a 100-Å Phenogel column using isooctane/ toluene/ acetic acid (65:35:0.15, v/v/v) as mobile phase. Detector: ELSD, column & injector temp: 60ºC .

- 31.29% WE
- 64.65% TG & DG
- 4.06% FFA

Experimental Setup



Dissolved catalyst (NaOH/ KOH) in anhydrous ethanol



CRBW: Ethanol = 1: 30, Room T.

Stopped reaction with glacial acetic acid

Washed

Mixing CRBW with anhydrous ethanol at RT

Phenogel Column (7.8x300 mm, 100-Å

Mobile phase: 0.15% acetic acid in isooctane: toluene (65: 35)

HPSEC-ELSD

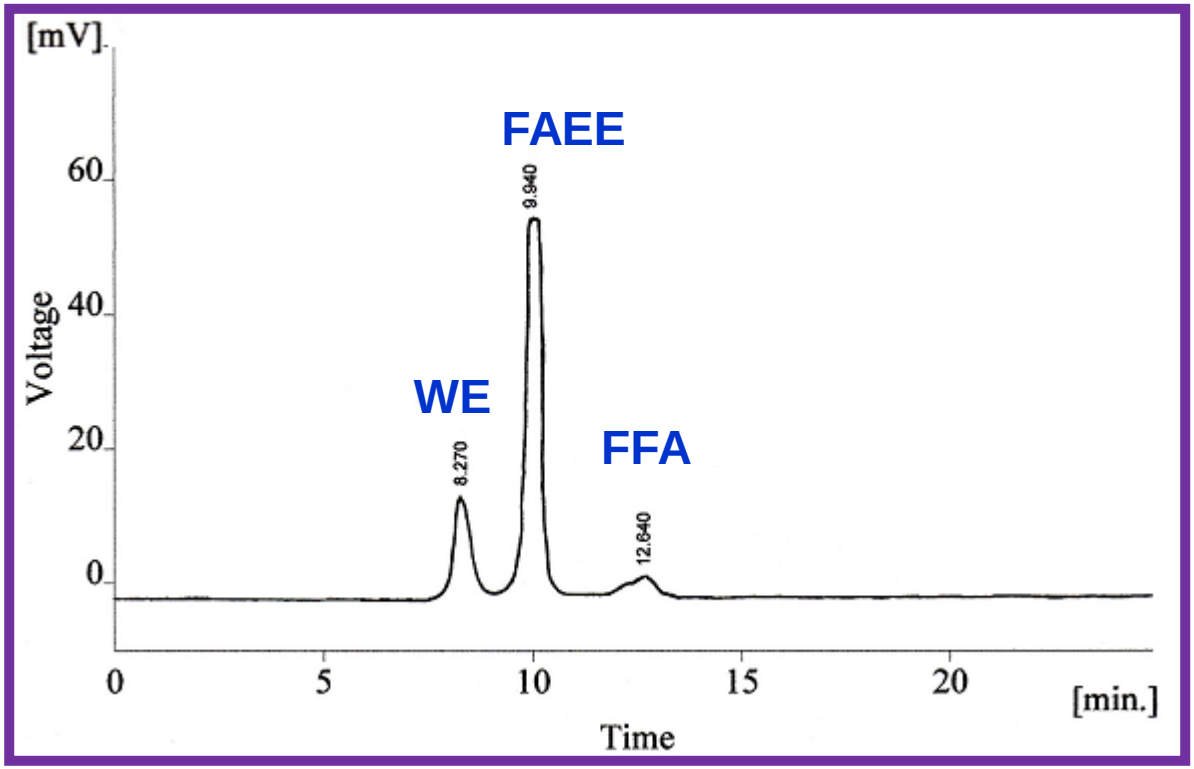


Fig. 3 HPSEC chromatogram of transesterified RBW using 1.6% NaOH as catalyst at 5 min.

Transesterification of CRBW by using NaOH

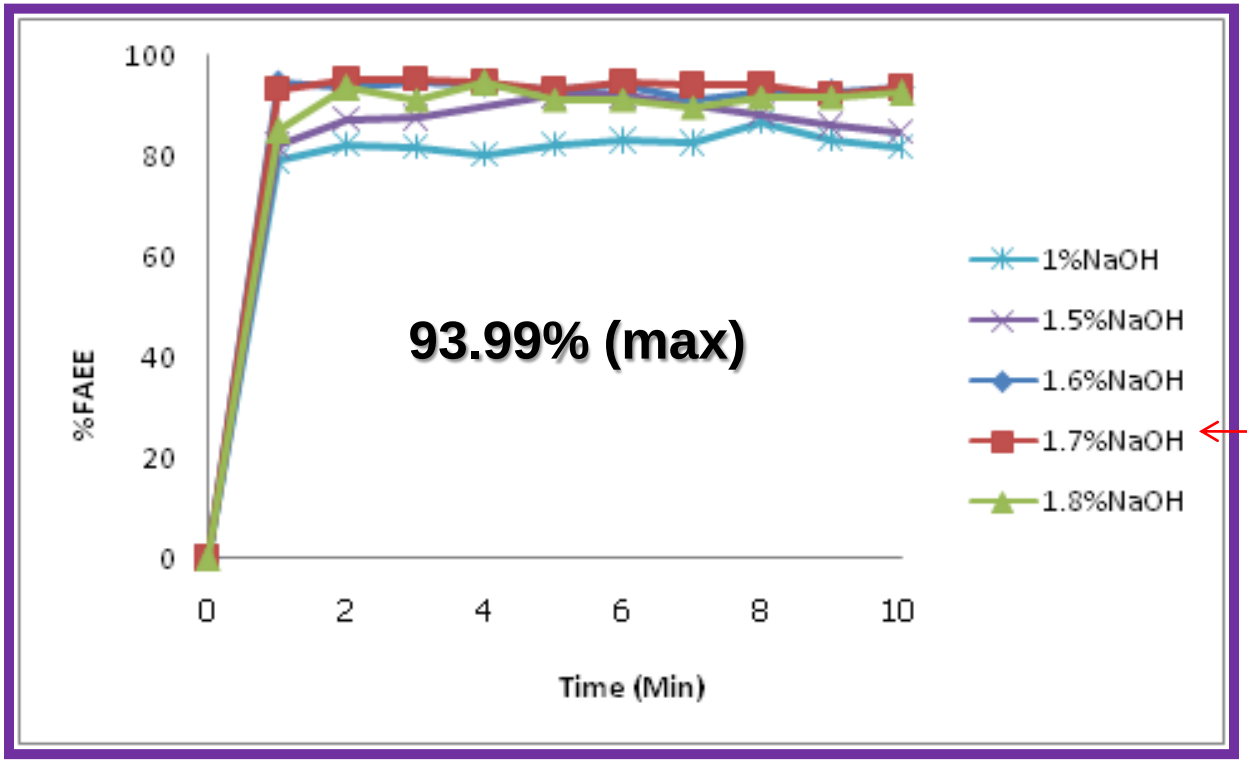


Fig. 4 The FAEE derived from transesterification of CRBW by using NaOH as catalyst at room temperature, molar ratio of CRBW: ethanol = 1: 30.

Transesterification of CRBW by using KOH

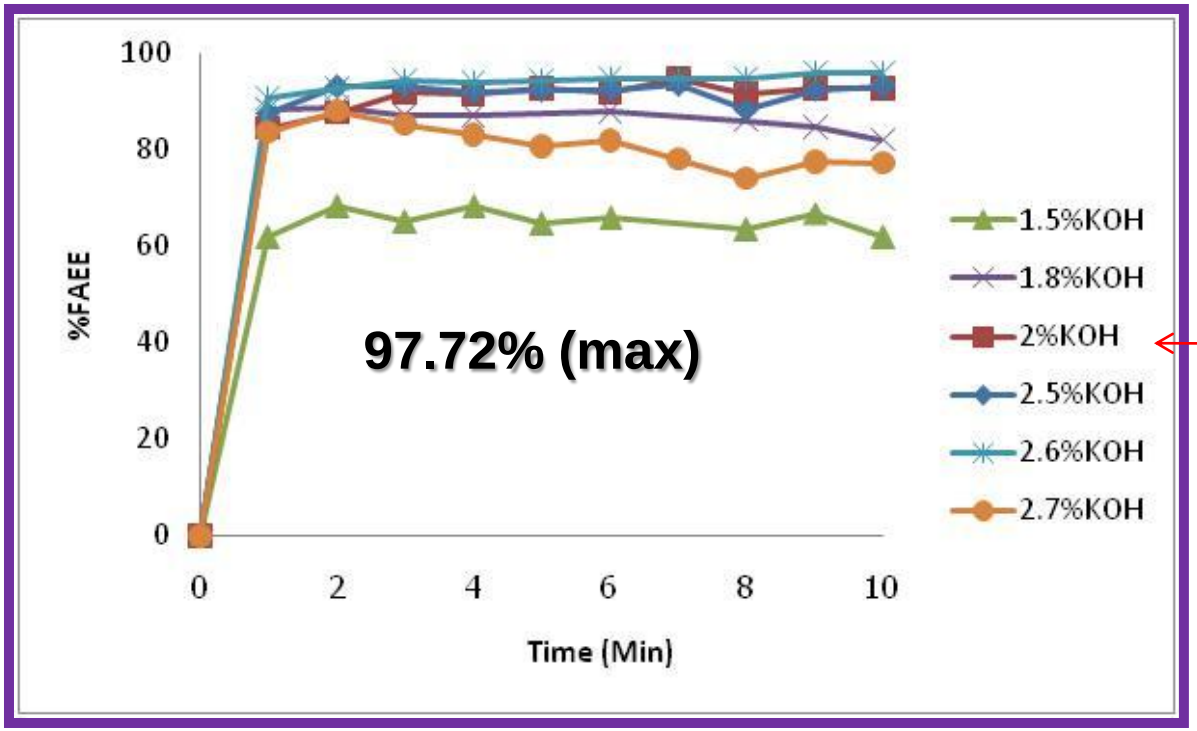
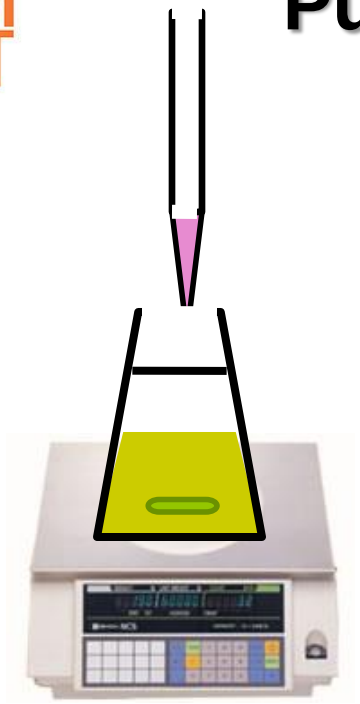
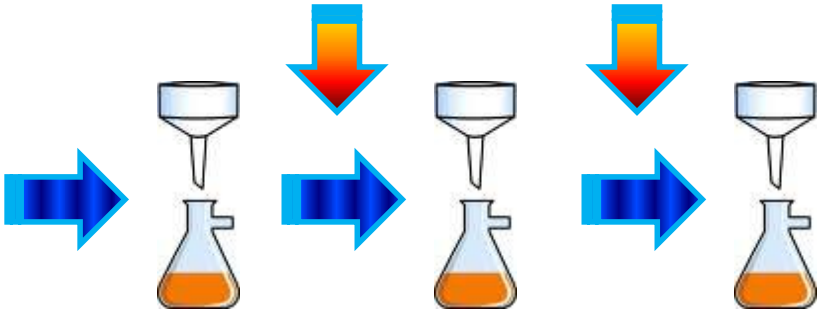


Fig. 5 The FAEE derived from transesterification of CRBW using KOH as catalyst at room temperature, molar ratio of CRBW: ethanol = 1: 30.

Purification of transesterified RBW



Fresh Ethanol



dried in hot air
oven at 50°C, 3 h

PRBW

FAEE

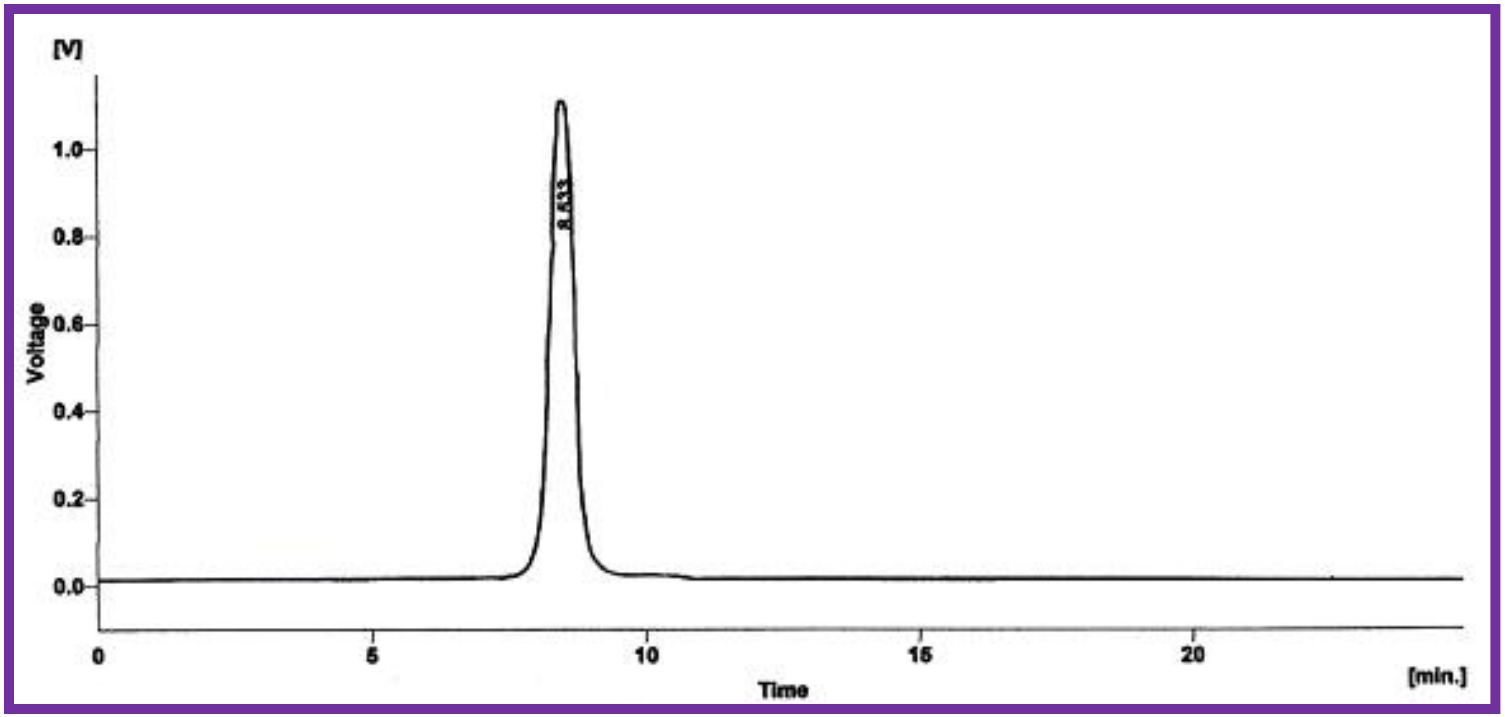


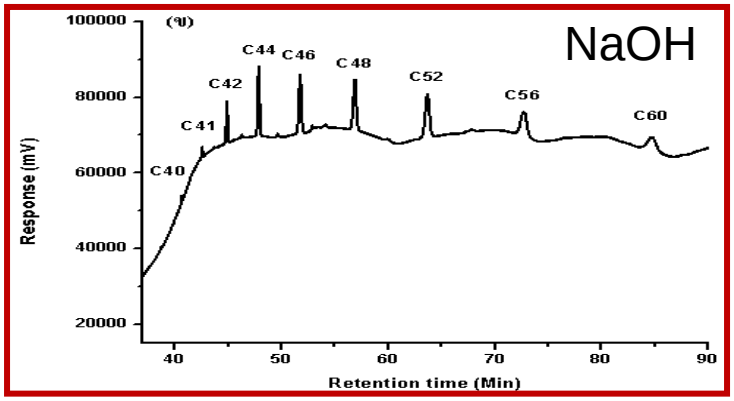
Fig. 6 HPSEC chromatogram of PRBW (WE) after being washed twice with fresh ethanol.

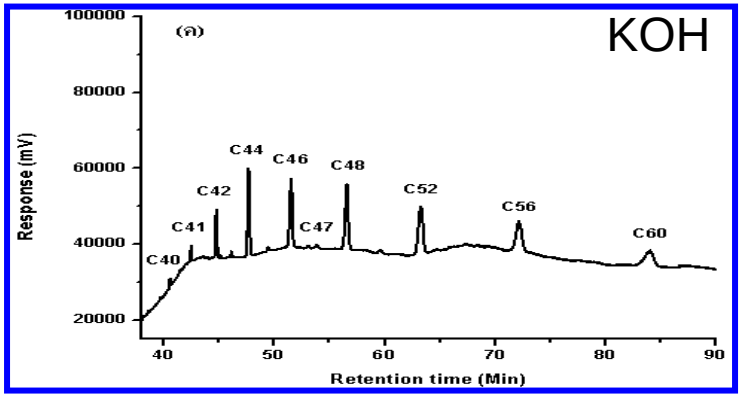
Fig. 7 The PRBW (WE) obtained from selective transesterification by using NaOH and KOH as catalyst.

Table 1 Physical and chemical properties of PRBW

Properties	FDA specification	PRBW (NaOH)	PRBW (KOH)
Mp (°C)	75-80	79-80	79-80
IV	20 (max)	6.5	6.5
SN	75-120	85	85
FFA (%)	10 (max)	1.5	1.5
Appearance		Free- flowing solid	Free- flowing solid
Color		Off-white	Off-white
Particle size (μm)		63-106	63-106

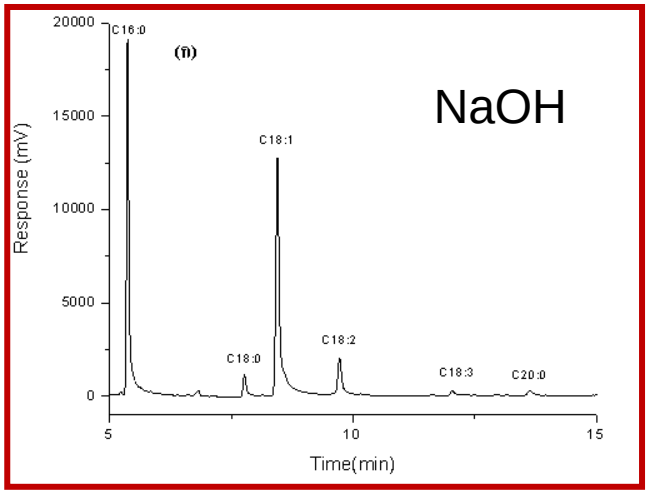
Table 2 The compositions of rice bran WE determined by HT-GC (column: ZB-5; detector: FID; inj.& det. temp.: 360ºC; column temp.: 150ºC 1.5 min rise 5ºC-350ºC hold 50 min.)

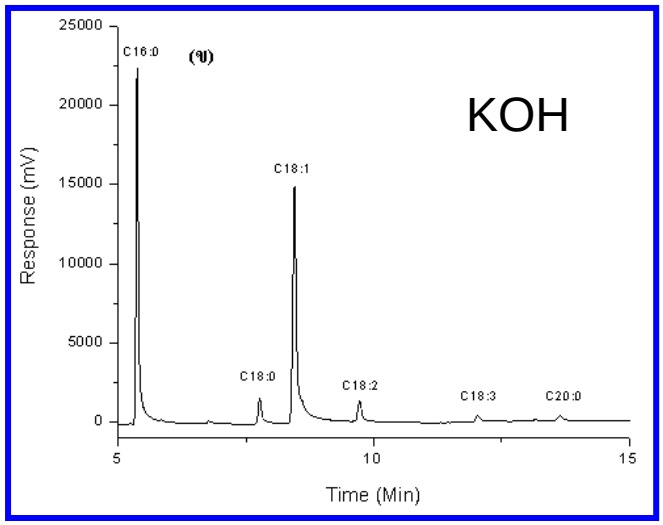




Carbon number	WE (%)	
	NaOH	KOH
40	1.77	3.40
41	10.95	10.21
42	5.82	5.84
44	12.34	12.19
46	13.54	13.75
48	15.76	15.29
52	17.34	16.87
56	14.03	13.51
60	8.44	8.94

Table 3 The compositions of rice bran FAEE determined by GC (column: BPX-70 ; detector: FID; inj.& det. temp.: 360ºC; column temp.: 180ºC)





Carbon number	FAEE (%)		
	NaOH	KOH	Refined RBO
C16:0	43.43	43.91	21.41
C18:0	3.55	4.24	1.18
C18:1	43.53	45.89	41.41
C18:2	7.23	3.97	35.50
C18:3	0.94	0.83	0.30
C20:0	1.32	1.17	0.20

Table 4 Some properties of fatty acid ethyl ester (FAEE).

Properties	Fatty acid ethyl ester			
	Std. * biodiesel	NaOH	KOH	Refined RBO
Iodine Value (IV)	120 max	49.76	46.06	93.02
Cetane Index (CI)	51 min	70.5	71.5	60.4
Kinematic viscosity at 40°C (cSt)	3.5-5.0	18.95	19.79	4.7

* EN 14214

Conclusion

- Both NaOH and KOH could be used as catalyst for selective transesterification of CRBW to prepare PRBW
- The transesterification of glycerides at RT was rapid and completed within 5 min with high molar ratio of the ethanol to wax.
- The wax ester and FAEE greatly differ in solubility and could be easily separated by simple filtration.
- The great advantages of the proposed method are
  - Reduction in solvent consumption
  - The reaction occur at room temperature
  - Easy separation
  - Greatly shorten the purification time

Up-scale Production

- Batch or continuous reactor may be applied

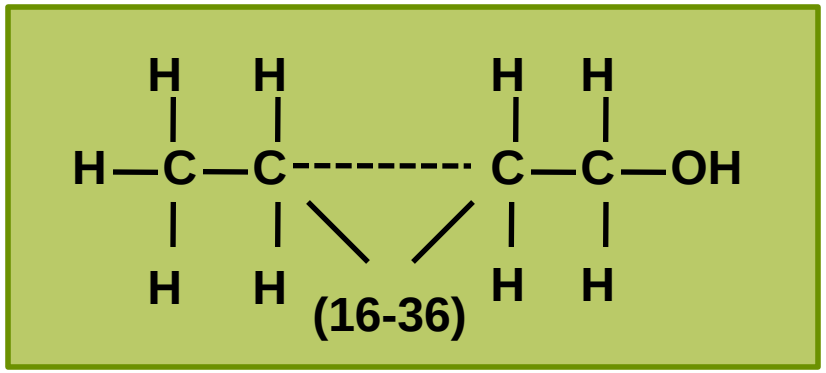
100 liter reactor

16 Kg, CRBW + 27.6 L, Ethanol
+ 469.2 g, NaOH, RT, 10 min

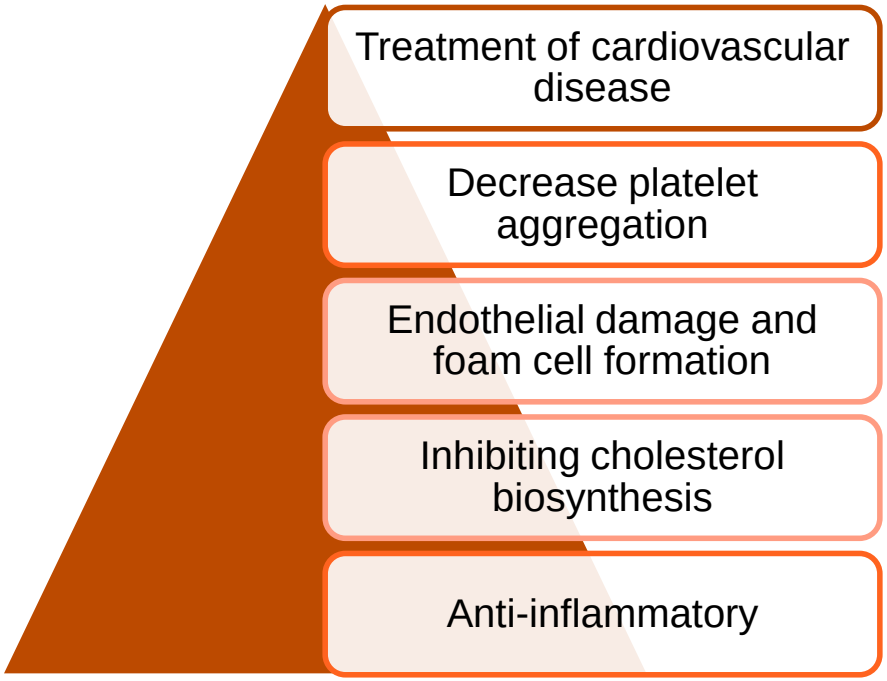
Plate & Frame Filter

Policosanol

- Mixture of long chain aliphatic primary alcohols
- Extracted from waxy materials of animals and plants



Health benefits of policosanol



- Irmak, *et al*, 2006 "Policosanol contents of beeswax, sugar cane and wheat extracts“, *Food Chem*, 95: 312-318.
- Granja, et al, 1999, "*Mixture of higher primary aliphatic alcohols, its obtention from sugar cane wax and its pharmaceutical uses* ", US Patent 5856316

Policosanol maintains excellent stability in

- Hair-, nail and skin-care formulations, and

- Delivers antimicrobial, emollient and sebum-regulating properties

- Majeed, et al., 2007, "*Compositions and methods containing high purity of fatty alcohol C_{24} to C_{36} for cosmetic applications*", US Patent 2007/0196507 A1

- Majeed, et al., 2007, "*Commercially viable process for high purity of fatty alcohol C_{24} to C_{36} and its cosmetic application for skin hair and nails* ", US Patent 7,217,546 B1

Production of Policosanol

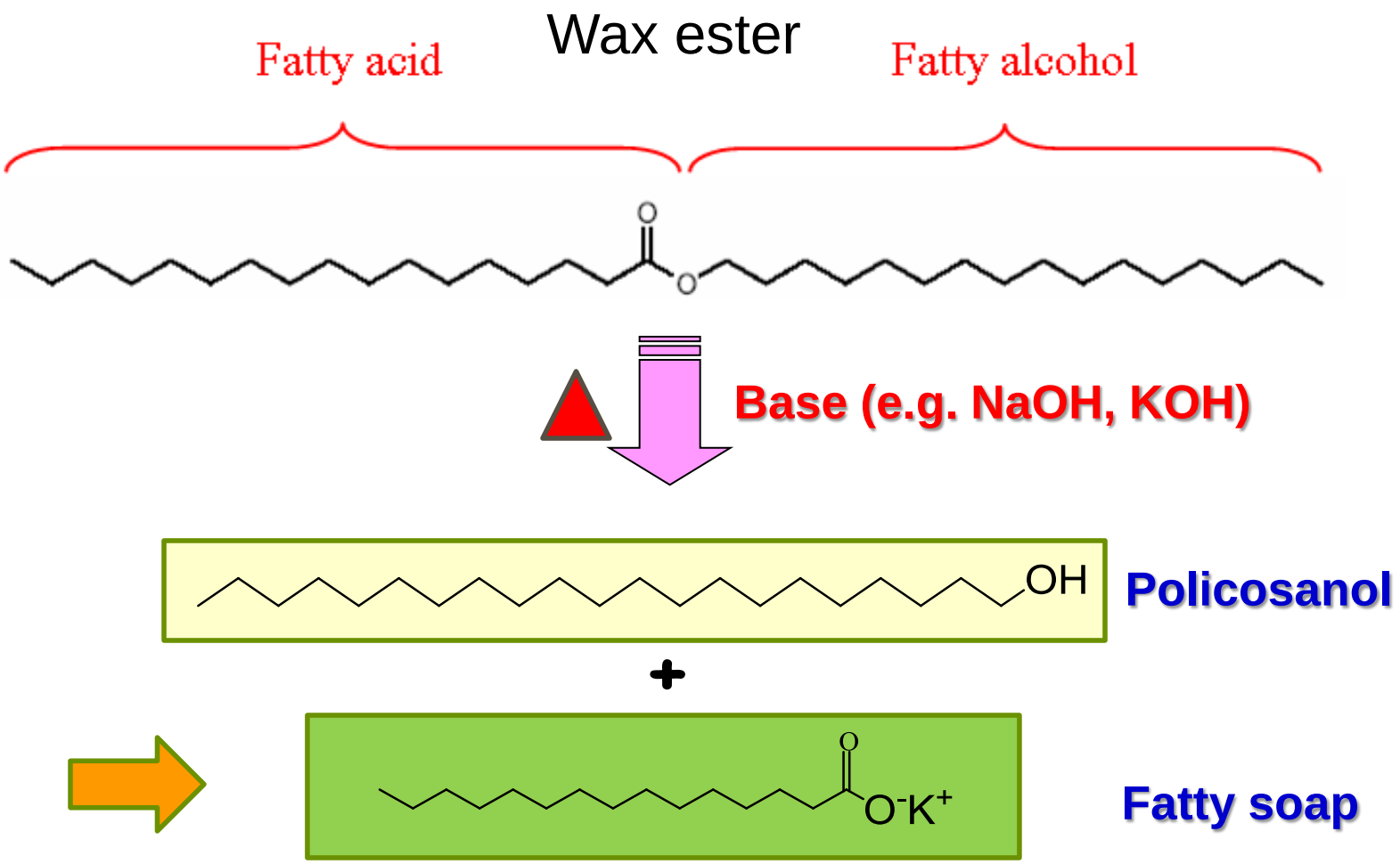
Step 1: Releasing policosanol from wax ester

- Saponification
- Transesterification

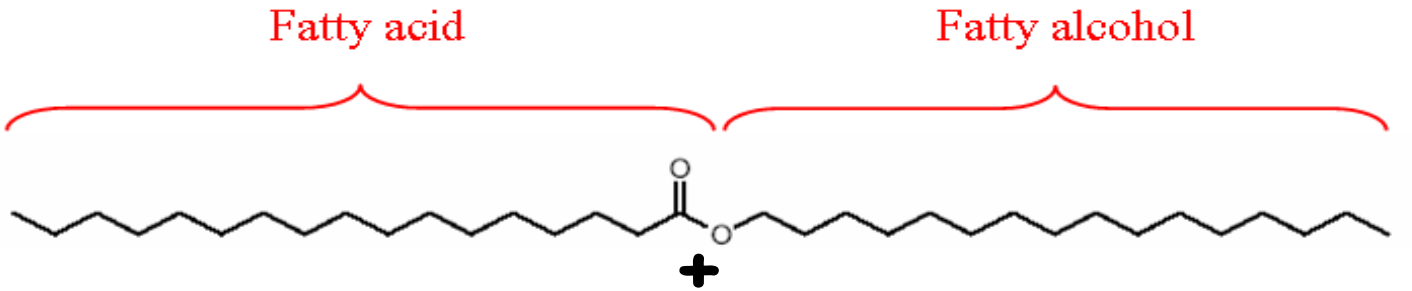
Step 2: Purification of policosanol

- Solvent extraction
- Crystallization

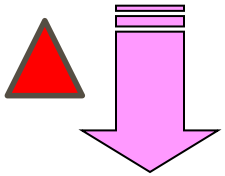
Saponification



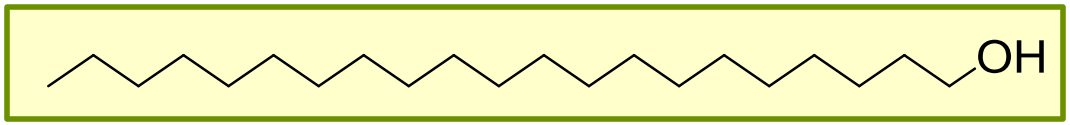
Transesterification



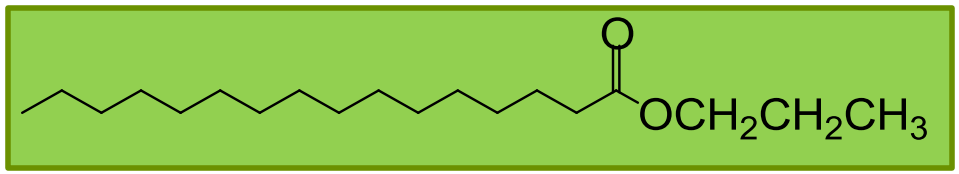
Alcohol (e.g. ethanol/ propanol)



Acid/ basic catalyst



Policosanol



FA propyl ester

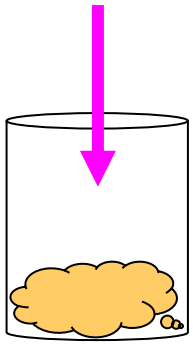
The Present Study

To compare the methods for splitting the policosanol from rice bran WE:

- Extraction time
- Composition
- Yield

Preparation of policosanol by saponification

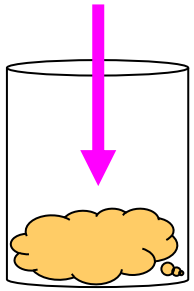
0.1% KOH in 90% Ethanol, 250 ml



50 g Rice bran WE

Refluxing T, 2 h

Hot water 200 ml
Isooctane 500 ml
Ethanol 70 ml

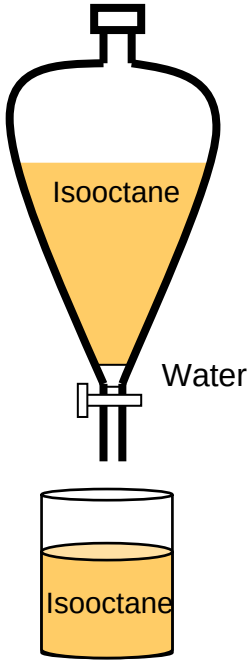


Saponified Wax

Stirred at refluxing temp., 1 h.

X 3-4

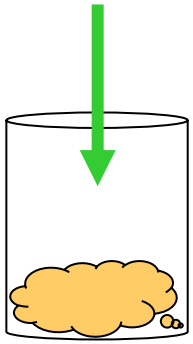
Solvent Extraction



Crystallization at 4°C for 18 h, Filtration

Preparation of policosanol by transesterification

0.5% NaOH in absolute ethanol, 250 ml

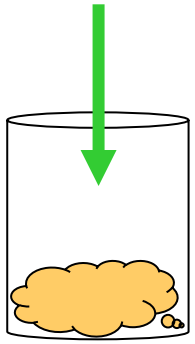


50 g Rice bran WE

Refluxing, 15 min

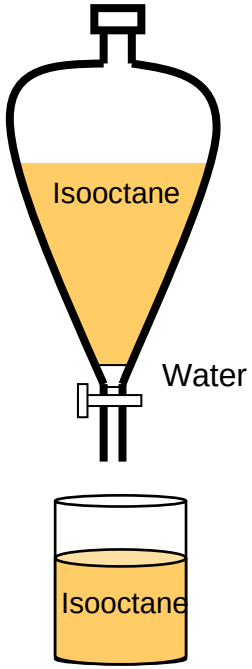
- Stop reaction with acetic acid

Isooctane 200 ml



Transesterified wax

Stirred



Crystallization at 4°C for 18 h, Filtration

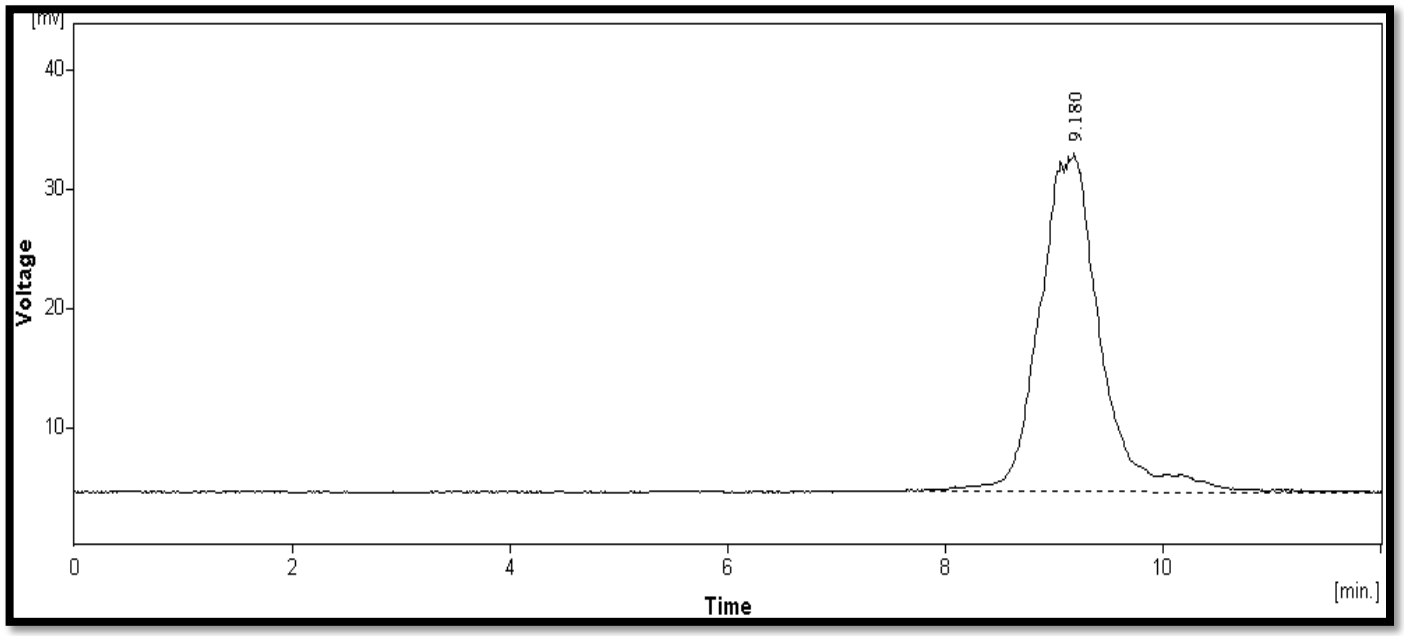


Fig. 8 HPSEC chromatogram of policosanol separated on a 100-Å Phenogel column using 0.25% acetic acid in toluene as mobile phase.

Acetylation of policosanol

- Policosanol was acetylated with ethyl acetate by using NaOH as catalyst in micro-reactor as described by Kaewkool and Krisnangkura.

GC Analysis

- Policosanol acetate was analyzed on GC-17A equipped with FID and split/splitless injector by using BPX35 column (35% Phenyl polysilphenylene-siloxane; 0.25 mm., ID. X 30 m.,L x 0.25 mm., d_f)
- Column temperature was set at 200°C x 2 min then increased to 350°C at 4°C/min; injector/detector, 360°C.

Kaewkool, P. and Krisnangkura, K., 2010, "Transesterification/acetylation of long chain alcohols with alkyl acetate," Chem Physics Lipids, 163: 685-688.

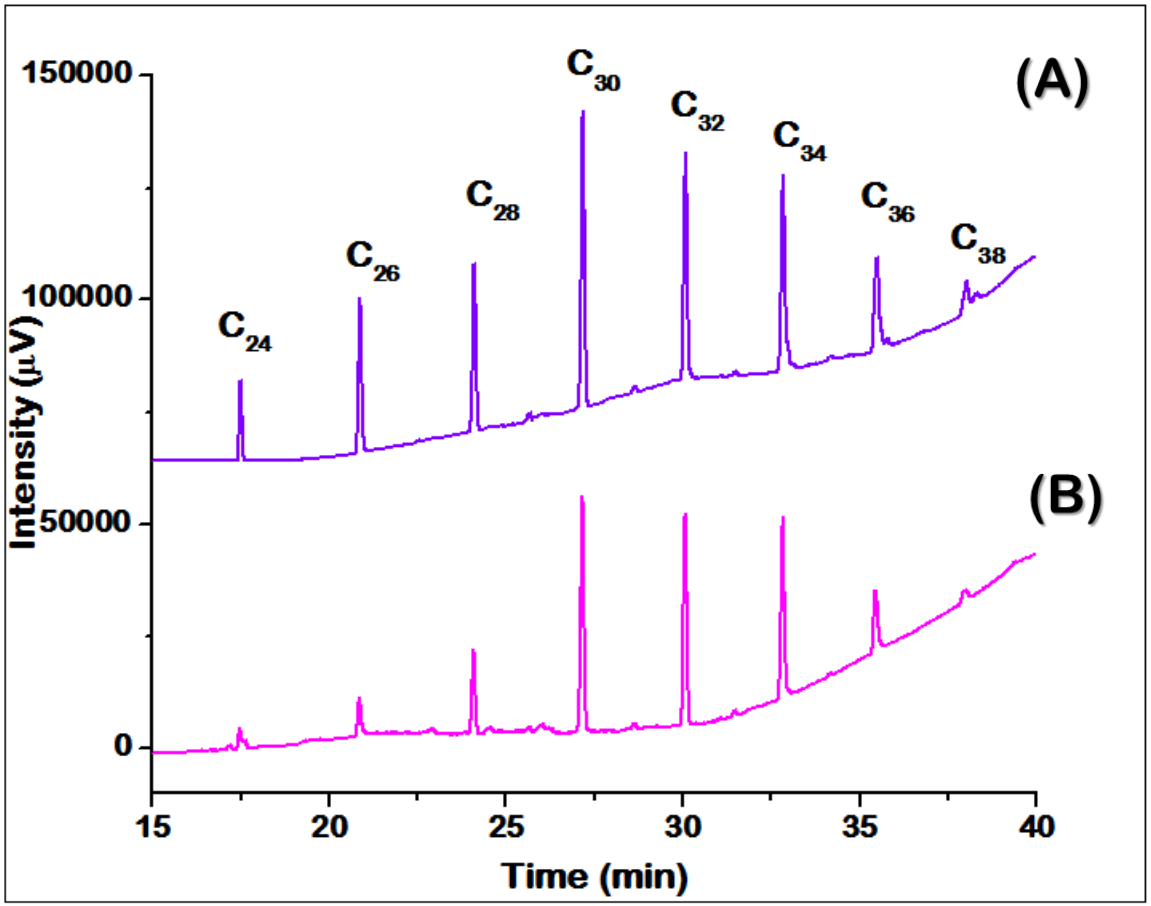


Fig. 9 GC chromatogram of policosanol prepared by saponification (A) and transesterification (B).

Table 4 Comparison of policosanol prepared by saponification and transesterification.

Fatty alcohol	Content (%)	
	Saponification	Transesterification
Tetracosanol (C_{24})	5.09	2.41
Hexacosanol (C_{26})	11.37	4.69
Octacosanol (C_{28})	12.81	9.76
Triacontanol (C_{30})	22.85	27.44
Dotriacontanol (C_{32})	17.44	24.38
Tetratriacontanol (C_{34})	17.03	22.08
Hexatriacontanol (C_{36})	10.29	8.45
Octatriacontanol (C_{38})	3.13	0.79
% Yields ($Yield = 100 \frac{w_{policosanol}}{w_{wax}}$)	3.2	31.45
% Purity	98.2	95
Extraction & purification time (h)	66	18

Conclusion

- Transesterification was more effective for releasing of policosanol from RBW than saponification. (2 h *vs*. 15 min reaction time)
- Gas chromatographic characterization of the policosanol as the acetate derivatives showed that the composition of policosanol prepared by transesterification was differ from that prepared by the widely accepted saponification method.
- The extraction of policosanol from the transesterification reaction medium was much simpler and gave higher yield.

CRBW

Transesterification → Purification

Policosanol

Acknowledgement

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E-mail: kornkanok.ary@kmutt.ac.th