Appendix

1. Acid value (AOAC, 1999)

A. Definition and Principle

Acid value of fat = mg KOH required to neutralize 1 g fat. Fat separated by melting butter, is dissolved in alcohol-ether mixture, and titrated with standard alkali.

B. Reagents

(a) *Alcoholic potassium hydroxide standard solution*. –0.1 *N*. Use absolute alcohol denatured with methanol.

(b) *Alcohol-ether mixture*.-Equal volumes alcohol (or alcohol denatured with methanol) and ether. Add 0.3 mL phenolphthalein solution, (c)/100 mL mixture and add alcoholic KOH solution, (a), to faint pink.

(c) *Phenolphthalein solution*. –1 % in alcohol or alcohol denatured with methanol.

C. Determination

Weigh, to the nearest mg, 5-10 g well-mixed sample into 250-300 mL Erlenmeyer. Add 50-100 mL alcohol-ether mixture and 0.1 mL phenolphthalein solution. Titrate with 0.1*N* alcoholic KOH until permanent faint pink appears and persists for $\geq$ 10 s.

Acid value = mL alcoholic KOH solution x normality alcoholic KOH solution x 56.1 /g sample. Difference between duplicate determinations should be $\leq$ 0.1 mg KOH/g fat.

2. Iodine value (AOAC, 1999)

A. Reagents

(a) Potassium (KI) solution. –15 %. Dissolve 15 g KI in 100 mL H_2O.

(b) Wijs iodine solution. – (1) Dissolve 13 g resublimed I in 1 L acetic acid, and pass in dried (through H_2SO_4) Cl until original $Na_2S_2O_3$ titration of

solution is not quite doubled. (Characteristic color change at the end point indicates proper amount of Cl. Convenient method is to reserve some of original I solution, add slight excess of Cl to bulk of solution, and bring to desired titer by readditions of reserved portion.) Or: (2) Dissolve 16.5 g ICl in 1 L acetic acid.

Determine I/Cl ratio as follows:

(1) Iodine content. – Pipet 5 mL Wijs solution into 500 mL Erlenmeyer flask containing 150 mL saturated Cl-H_2O and some glass beads. Shake, heat to boilling, and boil briskly 10 min. Cool, add 30 mL H_2SO_4 solution (1+49) and 15 mL 15% KI solution, and titrate immediately with 0.1*N* $Na_2S_2O_3$.

(2) Total halogen content. – Pipet 20 mL Wijs solution into 500 mL Erlenmeyer flask containing 150 mL recently boiled and cooled H_2O and 15 mL 15 % KI solution. Titrate immediately with 0.1 *N* $Na_2S_2O_3$.

$$I/Cl = 2X/(3B-2X)$$

where X = mL 0.1 *N* $Na_2S_2O_3$ required for I content and B = mL required for total halogen content. If I/Cl ratio is not 1.10$\pm$0.1, add I or Cl to correct ratio.

Standardized Wijs solution may be obtained from commercial suppliers (specify without carbon tetrachloride).

Store in amber bottle sealed with parafin until ready for use. Wijs solution are sensitive to temperature, moisture, and light. Store in dark at <30 °C.

(c) Soluble starch solution.- Mix paste of 1 g starch with small amount cold H_2O. While stirring, add 200 mL boiling H_2O. Test for sensitivity : place 5 mL starch solution in 100 mL H_2O and add 0.05 mL 0.1 *N* iodine solution; deep blue color produced must be dischanged by 0.05 mL 0.1 *N* sodium thiosulfate solution.

(d) Potassium dichromate ($K_2Cr_2O_7$). –Finely grind and dry to constant weight (ca 110) befor using in B.

(e) Sodium thiosulfate ($Na_2S_2O_3.5H_2O$) solution. – 0.1 *N* Standardize as in B.

(f) Acid. –(1) Hydrochloric acid (HCl). –Concentrated, (2) Acetic acid ($C_2H_4O_2$). (3) Sulfuric acid (H_2SO_4). –Concentrated.

(g) Cyclohexane.

(h) Cyclohexane-acetic acid solvent. – Mix cyclohexane, (g), and acetic acid, (f) (2), 1+1 (volume/volume). Verify absence of oxidizable matter in solvent by shaking 10 mL solvent with1 mL saturated aqueous $K_2Cr_2O_7$ solution and 1 mL H_2SO_4, (f)(3). No green color should appear.

B. Standardization of Sodium Thiosulfate Solution

Accurately weigh 0.16-0.22 g dried, finely ground $K_2Cr_2O_7$, A(d), to nearest 0.0001 g into 500 mL flask, dissolve in 25 mL H_2O, add 5 mL HCl, A(f) (1), and 20 mL KI solution, A(b), and rotate to mix. Let stand 5 min. Add 100 mL H_2O. Titrate with sodium thiosulfate solution, A(e), shaking continuously until yellow color has almost disappeared. Add 1-2 mL starch indicator solution, A(c), and continue adding thiosulfate solution slowly until blue color just disappears.

$Na_2S_2O_3$ solution normality, N = [20.394 x wt $K_2Cr_2O_7$]/mL sodium thiosulfate

D. Determination

Melt test sample, if not already liquid. Pass test sample through double layer of filter paper to remove any solid impurities and traces of H_2O. Sample must be absolutely dry.

Let filtered test sample cool to 68-71°C . Immediately weigh amount of test sample into clean, dry 500 mL flask

Prepare at least 2 blank determinations to run with each sample group.

Add 15 mL cyclohexane-acetic acid solvent, A(h), to each test sample and swirl to ensure that sample is completely dissolved.

Dispense 25 mL Wijs solution into flask containing test sample, stopper flask, and swirl to mix. Immediately set timer of 1.0 or 2.0 h, depending on iodine value of sample and store flasks in dark at 25±5 °C for duration of reaction.

Remove flasks from in dark, add 20 mL KI solution, A(b), and mix. Add 150 mL H_2O and gradually titrate with 0.1 *N* standard $Na_2S_2O_3$ solution, B, with constant and vigorous shaking or mechanical stirring. Continue titrating until yellow color has almost disappeared. Add 1-2 mL starch indicator solution to flasks and continue titrating until blue color has just disappeared.

E. Calculation

Iodine value (IV) = [(B-S) x *N* x 12.69] / wt of sample

where B= titration of blank (mL); S = titration of sample (mL); *N* = normality of $Na_2S_2O_3$ solution.

3. The Folin-Lowry Method (Lowry *et al*., 1951)

The copper reagent is prepared by adding 1.0 mL of 0.5 % copper sulfate pentahydrate and 1% sodium-potassium tartrate to 50 mL of 0.1 M sodium hydroxide, containing 2 % sodium carbonate. The protein solution (0.5 mL) is added to 5.0 mL of the alkaline copper reagent and the solution allowed to stand for 10 minutes. Then 0.50 mL of the Folin-Ciocalteu phenol reagent (which had been diluted 1:1 with water) is rapidly added and mixed. This solution is allowed to stand for 30 minutes, and the absorbance is measured at 750 nm. A standard curve is prepared using the protein under study or some other protein, such as serum albumin at concentrations of 20-400 μg/mL.

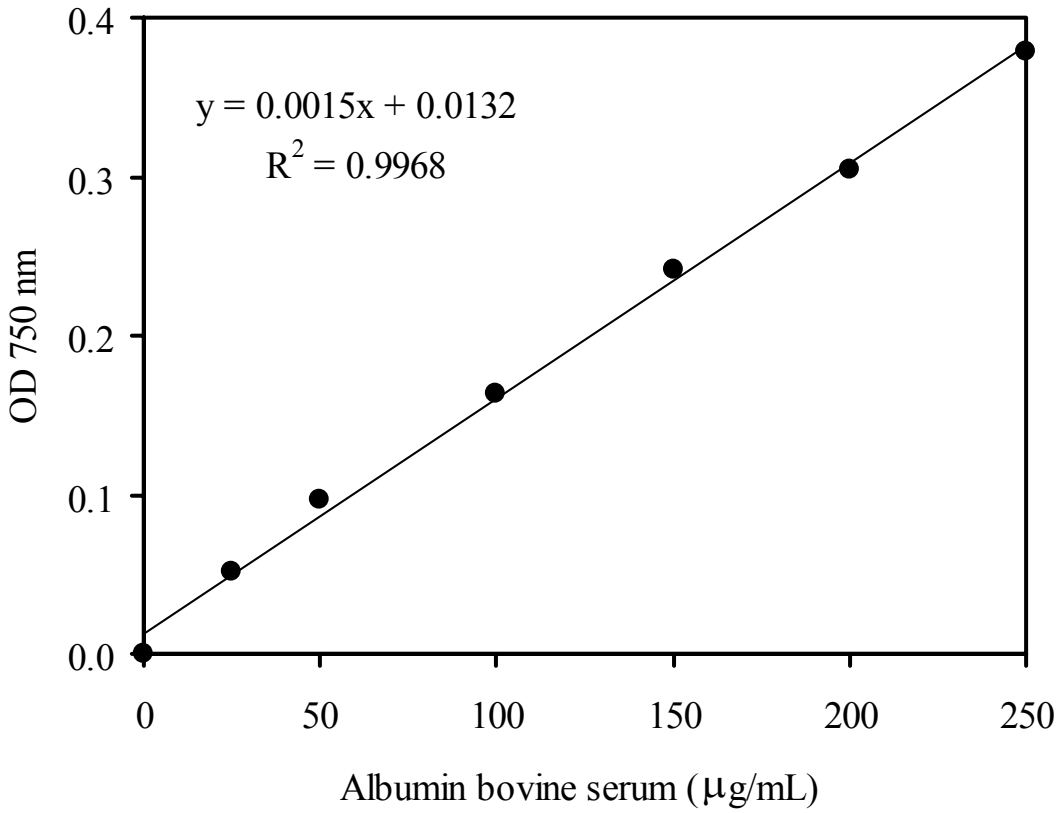


Figure 56 Standard curve of albumin bovine serum

4. Sponification value (AOAC, 1999)

A. Reagent

1. 0.5*N* Alcoholic potassium hydroxide solution
2. 0.5 *N* HCl
3. 1 % pheholphthalein

B. Determination

Accurately weigh ca 5 g filtered sample into 250-300 mL Erlenmeyer. Pipet 50 mL alcoholic KOH solution into flask, draining pipet definite time. Connect flask with air condenser and boil until fat is completely saponified (ca 30 min). Cool, and titrate with 0.5 *N* HCl., using phenolphthalein. Conduct

blank determination along with that on sample, using same pipet for measuring KOH solution and draining at the same time.

C. Calculation

Calculate saponification number = 28.05 (*B*-*S*)/g sample

(mg KOH required to saponify 1 g fat)

Where *B*= mL 0.5 *N* HCl required by blank and *S*= mL 0.5 *N* HCl required for sample

5. Hydrolytic activity of lipase (Lee and Rhee , 1993)

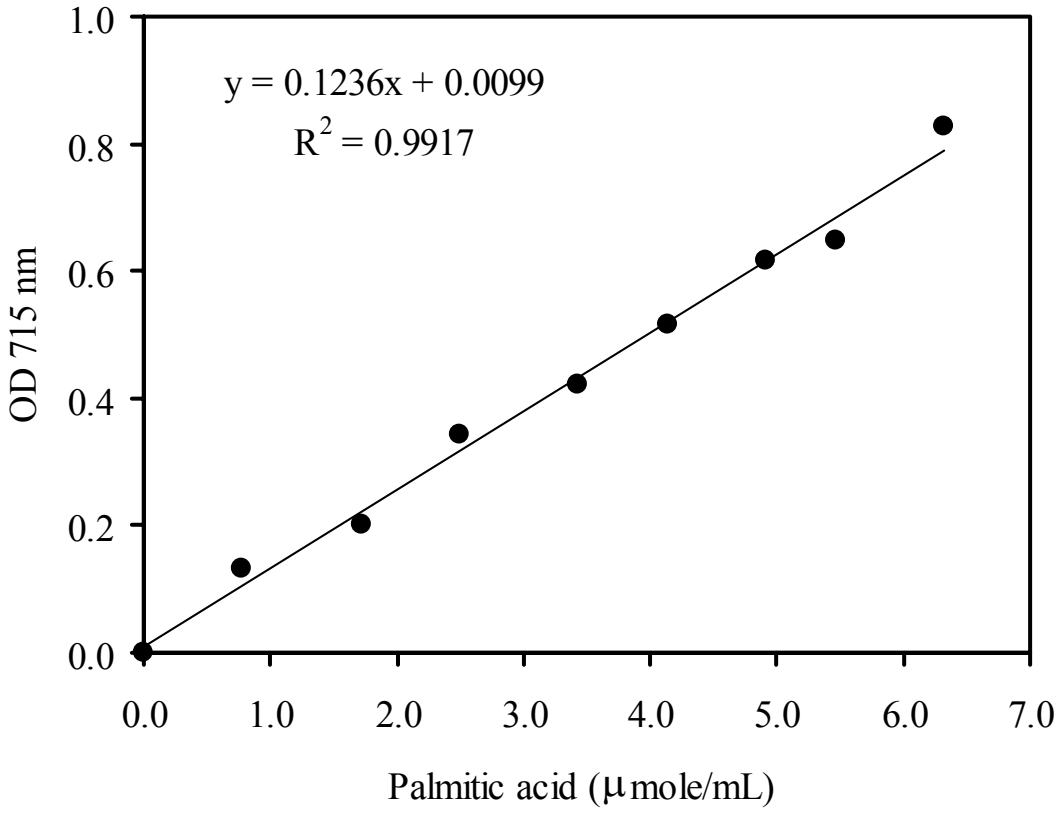
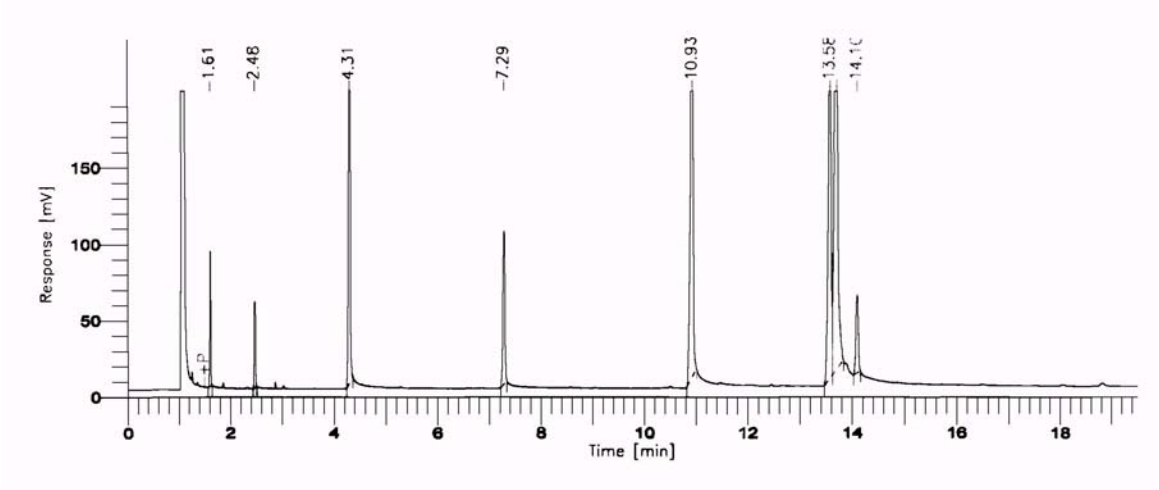


Figure 57 Standard curve of palmitic acid

6. Determination of fatty acid composition by GC analysis



Peak #	Name	Time (min)	Area (μV.s)	Height (μV)	Norm Area (%)	BL	Area (%)
1	Carylic acid	1.612	86533.16	90635.64	0.00	BB	1.91
2	Capric acid	2.475	69200.78	56301.07	0.00	BB	1.53
3	Lauric acid	4.306	640607.63	331053.52	0.00	BB	14.17
4	Myristic acid	7.286	229092.39	100011.80	0.00	BB	5.07
5	Palmitic acid	10.933	1024843.88	336347.75	0.00	BB	22.67
6	Linoleic acid	13.584	850922.51	222476.38	0.00	BV	18.82
7	Oleic acid	13.712	1458354.92	382037.32	0.00	VB	32.26
8	Stearic acid	14.101	161757.93	50822.36	0.00	BB	3.58
			4521313.19	1.57e+06	0.00		100.00

Condition:

Column : Optima-5-0.25 μm 25 m x 0.25 mm

Carrier gas : Helium at 1.65 mL/min

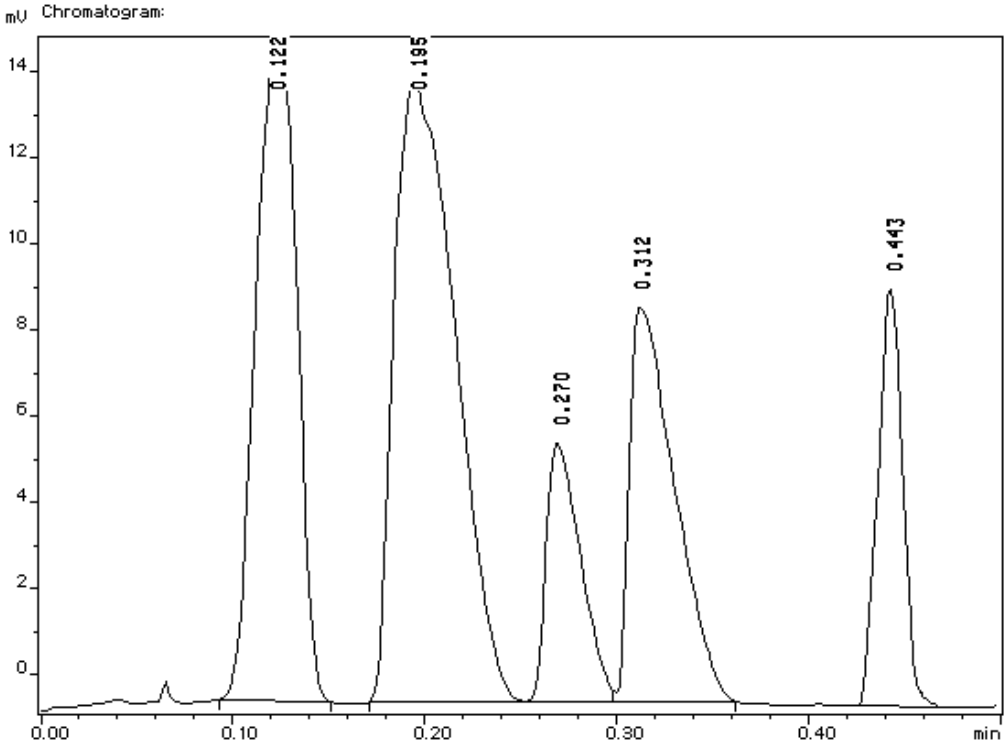
Temp : 150 °C (40 °C/min, 0.5 min), 170 °C (5°C/min), 195°C (10°C/min) and 215°C (9.5 min)

Injector temp : 250 °C

Detector : FID at 250 °C

Figure 58 GC chromatogram of standard fatty acid mixture

7. Determination of oil composition by TLC/FID analyzer



Peak No	Name	Ret. Time (min)	Pk. Start (min)	Pk. End (min)	Area	Height (mV)	Area %
1	Triolein	0.123	0.093	0.152	12059	15.26	26.731
2	Oleic acid	0.195	0.172	0.248	16658	14.41	36.923
3	1,3-Diolein	0.270	0.248	0.298	3913	5.99	8.674
4	1,2-Dioleoyl-rac-glycerol	0.313	0.298	0.362	7831	9.07	17.358
5	Monopalmitin	0.443	0.427	0.472	4654	9.67	10.315
					45115	54.39	100.000

Condition:

Stationary phase : CHROMAROD-SIII

Mobile phase : benzene : chloroform : acetic acid (50:20:0.7)

Gas flow : H_2 160 mL/min, Air 2.0 L/min

Scanning speed : 30 s/scan

Figure 59 TLC/FID chromatogram of standard oil compositions

Vitae

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Bachelor of Science (Agriculture)	Kasetsart University	1991
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Poster Presentations

Kaewthong, W., Prasertsan, P. and H-kittikun, A. 1999. Monoacylglycerols production from palm olein by immobilized lipase in continuous stirred tank and packed-bed reactor. The 5th Asia-Pacific Biochemical Engineering Conference 1999 and The 11 th Annual Metting of the the Thai Society for Biotechnology. Arcadia Hotel & Resort, Phuket, Thailand. 15-18 November 1999.

Kaewthong, W., Prasertsan, P. and H-kittikun, A. 2000. Monoacylglycerols Production from Palm Olein by Lipase in Continuous Stirred Tank with an In-Line Separation Column. The 12th Annual Meeting of the TSB "Biotechnology: Impacts & Trends". Felix Hotel, Kanchanaburi, Thailand. 1-3 November 2000.

Kaewthong, W., Prasertsan, P. and H-Kittikun, A. 2001. Monoglyceride Production by Glycerolysis of Palm Olein with Immobilized Lipase in Organic Solvents. BioThailand 2001 : From Research to Market. Queen Sirikit National Convention Center, Bangkok, Thailand. 7-10 November 2001.

Publication

Kaewthong, W. and H-Kittikun, A. 2001. Monoacylglycerols production with immobilized lipase. Songklanakarin J. Sci. Technol. 23(1):149-157.

Kaewthong, W., Sirisansaneeyakul, S., Prasertsan, P. and H-Kittikun, A. 2003. Continuous production of monoacylglycerols by glycerolysis of palm olein with immobilized lipase. Process Biochem. (in press)

Kaewthong, W. and H-Kittikun, A. 2003. Glycerolysis of palm olein by immobilized lipase PS in organic solvents. Enz. Microb. Technol. (submitted)