1. Hydrolytic activity of lipase on *p*-nitrophenyl ester (Kademi *et al*., 2000)

Hydrolytic activity of lipase solution was carried out by spectrophotometric method using *p*-nitrophenyl ester as substrate. The concentration of *p*-nitrophenol was measured at 410 nm. One unit of enzyme was the amount of enzyme liberating one μmol *p*-nitrophenol/mL/min under the determined conditions. The concentration of *p*-nitrophenol was calculated by Lambert and Bear equation:

$$A = \frac{\Delta E}{\varepsilon \times d \times c}$$

Where A = Activity (U/mL)

ΔE = Absorbance at 410 nm

ε = Molar extinction coefficient (L/mol/cm)

d = Cuvette width (cm)

c = Amount of enzyme (mL)

The molar extinction coefficient (ε) of *p*-nitrophenol was depended on pH value (Figure 69).

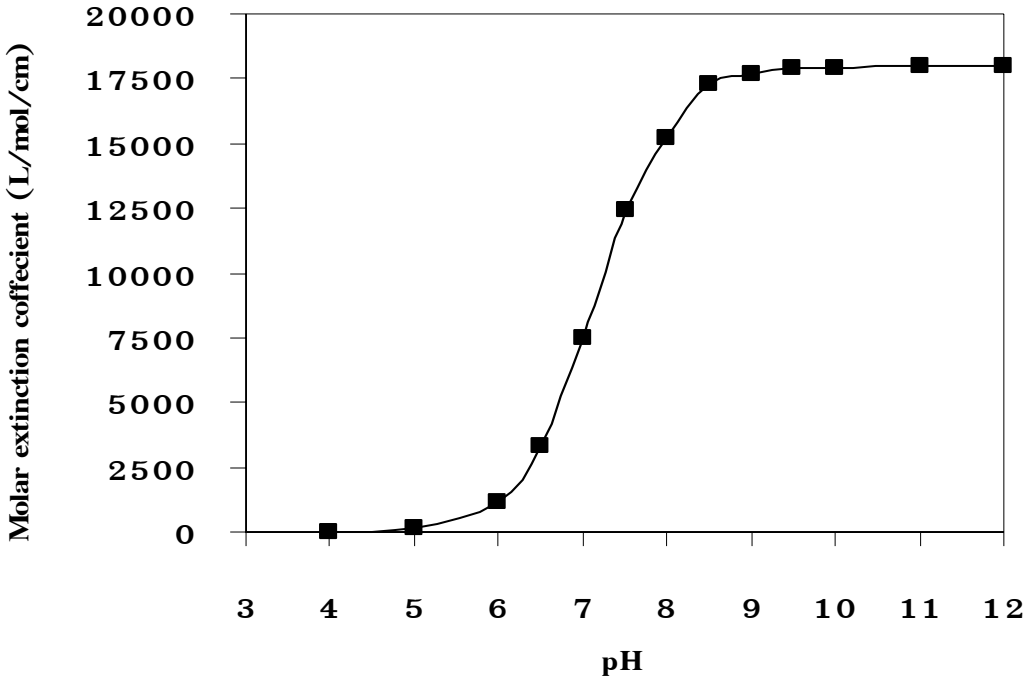


Figure 69. Calibrating curve of molar extinction coefficient of *p*-nitrophenol with different pH.

2. Hydrolytic activity of lipase by cupric acetate method (Lee and Rhee, 1993)

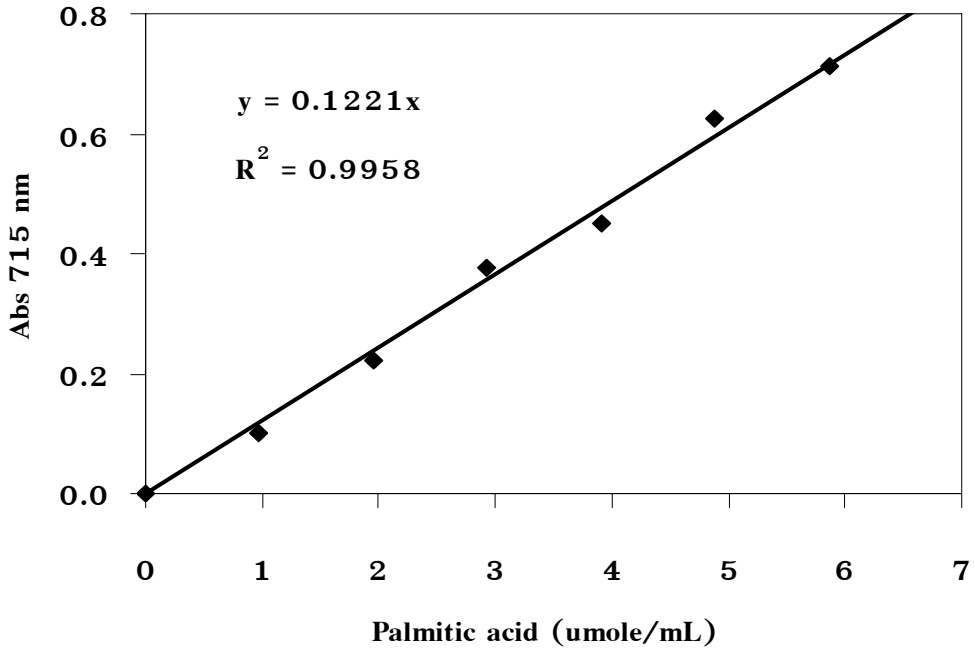
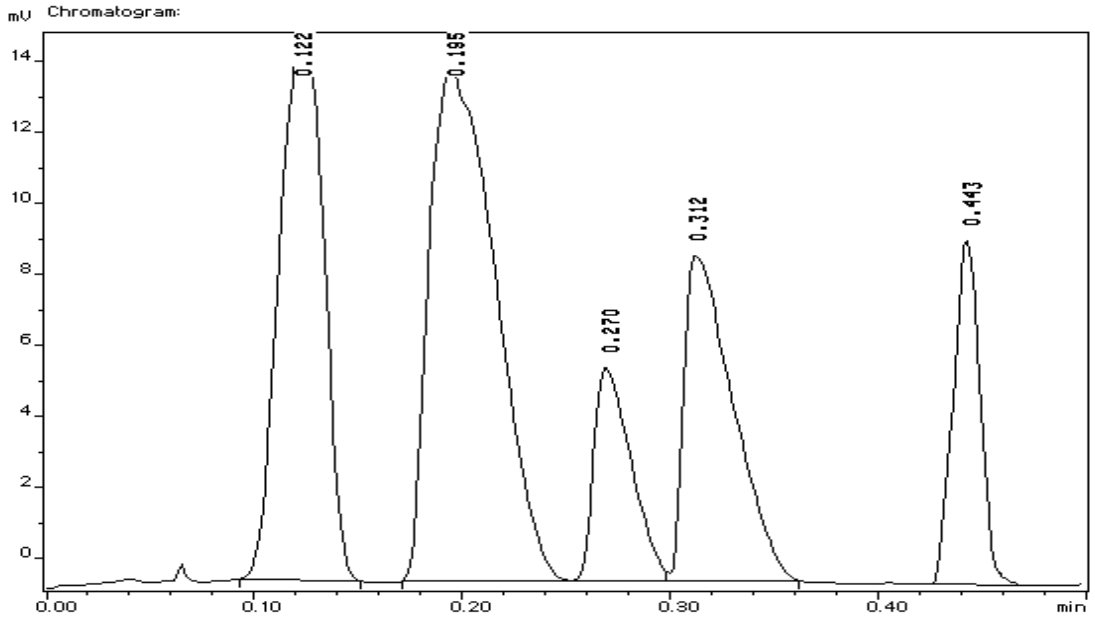


Figure 70. Standard curve of palmitic acid

3. TLC-FID chromatogram of standard oil



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	1-Monopalmitin	0.443	0.427	0.472	4654	9.67	10.315
					45115	54.39	100.000

Condition:

Stationary phase: CHROMAROD-SIII

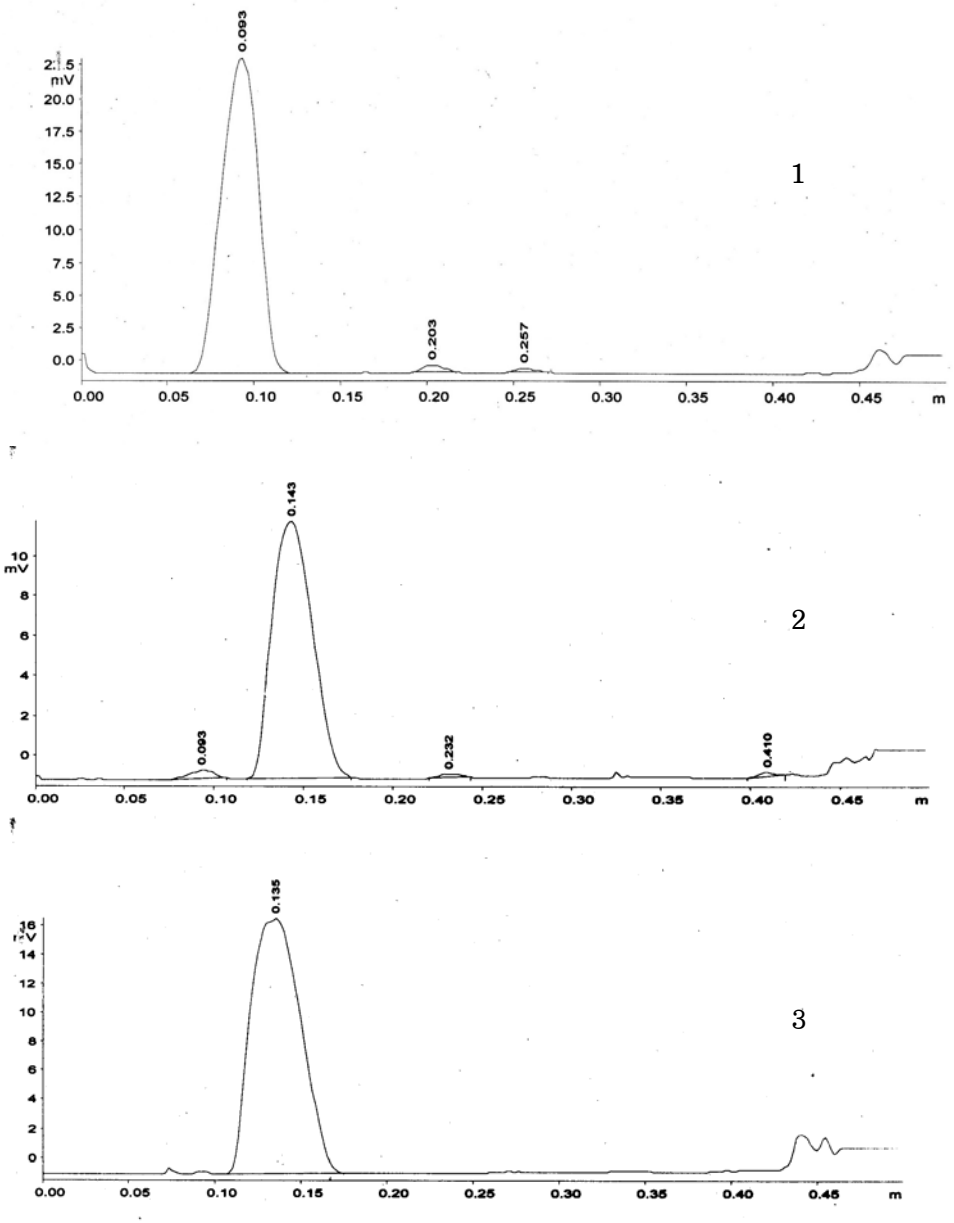
Mobile phase: benzene/chloroform/acetic acid (50:20:0.7)

Gas flow: H_2 150 mL/min, air 700 mL/min

Scanning speed: 30 sec/Rod

Figure 71. TLC-FID chromatogram of standard oil.

4. TLC-FID chromatogram of palm oil and PFAD

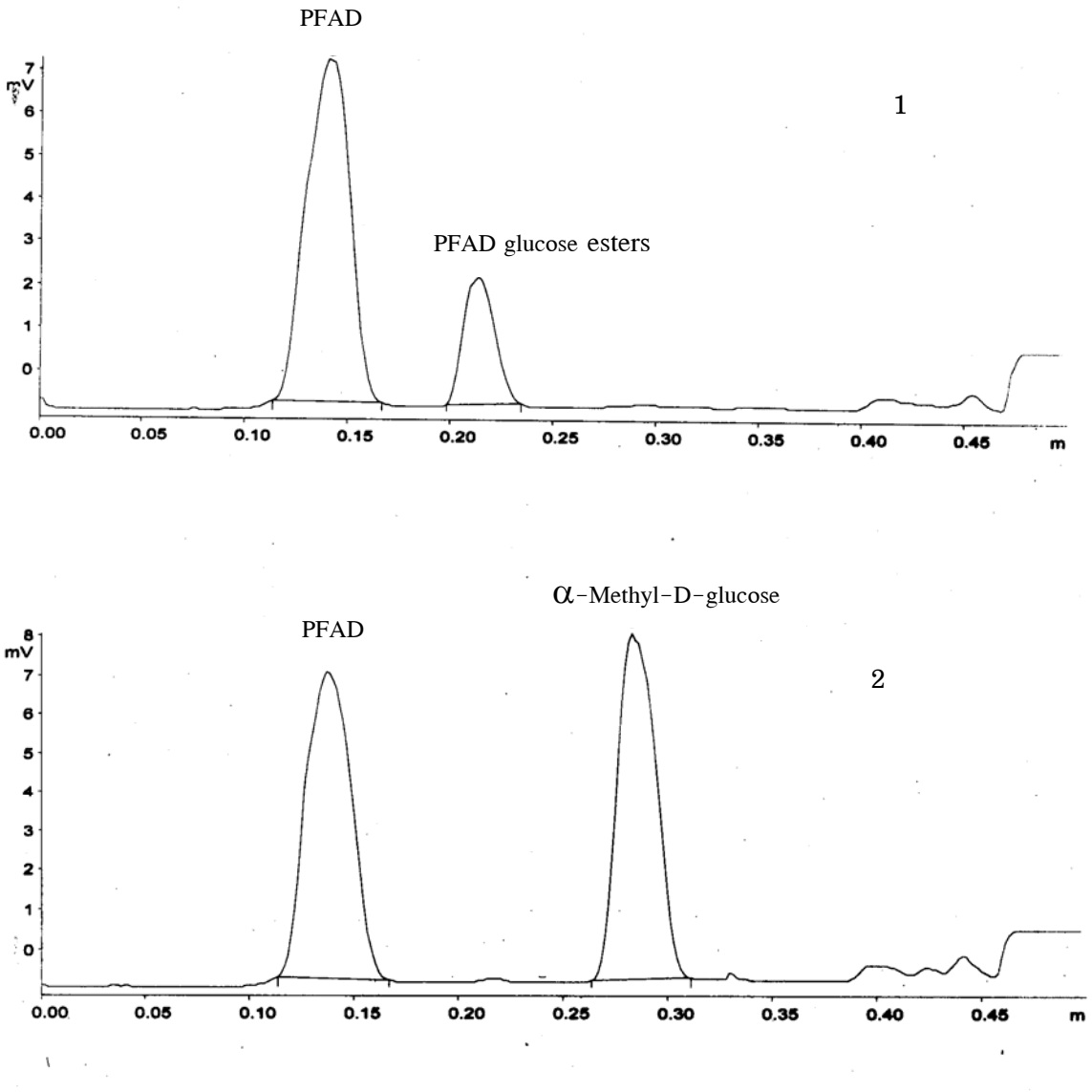


Condition:

Stationary phase:	CHROMAROD-SIII
Mobile phase:	Benzene/chloroform/acetic acid (50:20:0.7 v/v/v)
Gas flow:	H_2 150 mL/min, air 700 mL/min
Scainning speed:	30 sec/Rod

Figure 72. TLC-FID chromatogram of palm oil (1), crude PFAD (2) and partial purified PFAD (3).

5. TLC-FID chromatogram of PFAD glucose esters

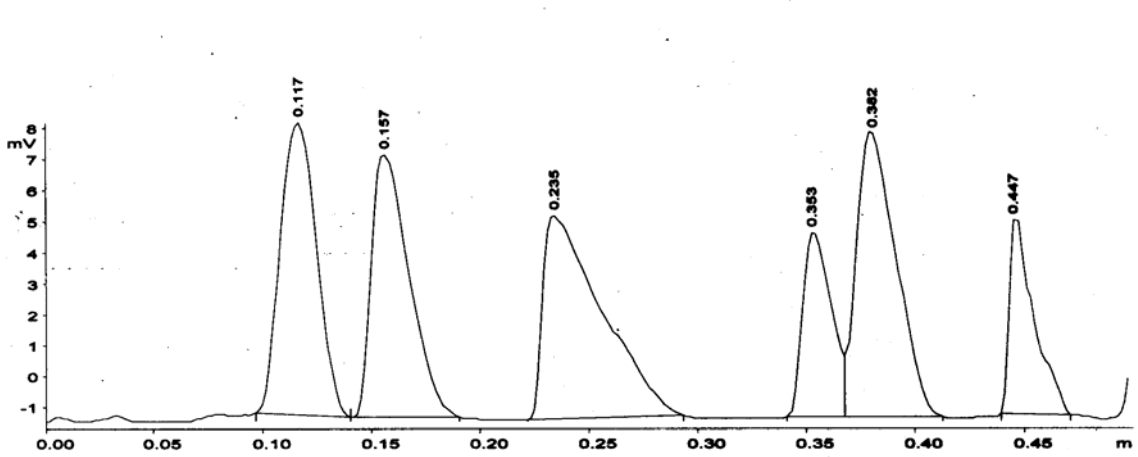


Condition:

Stationary phase:	CHROMAROD-SIII
Mobile phase:	Chloroform/methanol/formic acid (50:10:1 v/v/v)
Gas flow:	H_2 150 mL/min, air 700 mL/min
Scainning speed:	30 sec/Rod

Figure 73. TLC-FID chromatogram of reaction mixture of PFAD glucose esters (1) synthesis and α-Methyl-D-glucose as standard sugar ester (2).

6. TLC-FID chromatogram of fatty acid methyl esters (FAME)



Peak No	Name	Ret. Time (min)	Pk. Start (min)	Pk. End (min)	Area	Height (mV)	Area %
1	Palmitic acid methyl ester	0.117	0.097	0.140	5792	9.43	19.790
2	Triolein	0.157	0.140	0.190	5339	8.49	18.245
3	Oleic acid	0.235	0.222	0.293	6462	6.58	22.082
4	1,3-Diolein	0.353	0.342	0.368	3022	5.99	10.327
5	1,2-Dioleoyl-rac-glycerol	0.382	0.368	0.413	6117	9.16	20.901
6	1-Monopalmitin	0.447	0.440	0.472	2533	6.31	8.655
					29265	45.95	100

Condition:

Stationary phase: CHROMAROD-SIII

Mobile phase: Chloroform/diethyl ether/formic acid (50:20:0.7) for 15 min and benzene/hexane (50:50) for 35 min

Gas flow: H_2 150 mL/min, air 700 mL/min

Scanning speed: 30 sec/Rod

Figure 74. TLC-FID chromatogram of standard oil compositions and FAME.

7. Determination of fatty acid compositions by GC analysis

Table 30. Retention time of standard fatty acid methyl esters.

Fatty acid	Retention time (min)
Caprylic acid (C8:0)	1.63-1.68
Caproic acid (C10:0)	2.50-2.53
Lauric acid (C12:0)	4.33-4.36
Myristic acid (C14:0)	7.10-7.17
Palmitoleic acid (C16:1)	9.50-9.51
Palmitic acid (C16:0)	9.75-9.76
Linolenic acid (C18:3)	11.97-11.98
Linoleic acid (C18:2)	12.05-12.06
Oleic acid (C18:1)	12.15-12.18
Stearic acid (C18:0)	12.56-12.57
EPA (C20:5)	14.99-15.00
DHA (C22:6)	21.90-21.91
Behenic acid (C20:0)	22.30

Column OPTIMA-5 (25 m x 0.25 mm i.d.)

Condition T_1 = 150°C (4°C/min, 0.50 min)

T_2 = 170°C (10°C/min)

T_3 = 195°C (10°C/min)

T_4 = 215°C (15 min)

Injection temperature 250°C

Detection FID (250°C)

Carrier gas Helium (1.24 mL/min)

8. Protein determination by Bradford's method (Bradford, 1976)

Protein content of enzymes was determined by Bradford's method.

The Bradford reagent is prepared by 100 mg of comassie brilliant blue G 250 is dissolved in 100 mL ethanol. Then 100 mL of 85% phosphoric is added and mixed well. The volume is adjusted to 600 mL by distilled water and then mixed with 100 mL glycerol. The volume is adjusted to 1 L by distilled water and undissolved substrant is filtered out. The Bradford solution is kept overnight at 4°C before use.

To determine of protein concentration, 100 mL of sample is mixed with 5.0 mL Bradford's reagent. This solution is allowed to stand for 5 min and the absorbance is measured at 595 nm. A standard curve is prepared using the protein under study or some other protein, such as serum albumin at concentrations of 100-1000 μg/mL.

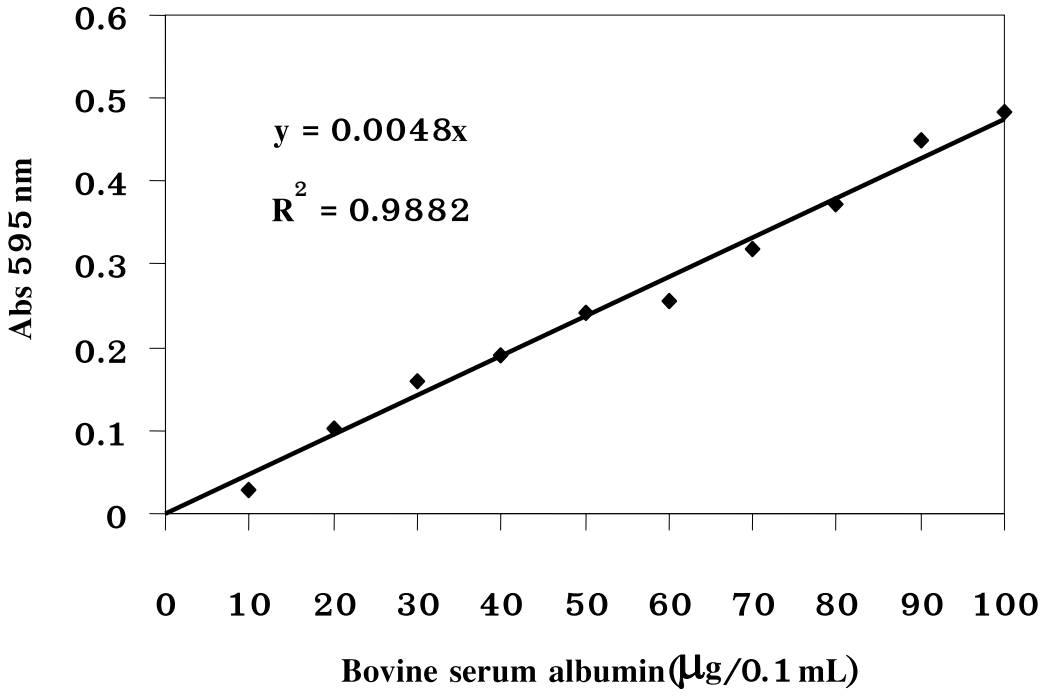


Figure 75. Standard curve of bovine serum albumin.

9. Saponification value (AOAC, 1999)

A. Reagent

1. 0.1 N Alcoholic potassium hydroxide solutin
2. 0.5 N HCl
3. 1% phenolphthalein

B. Determination

Accurately weight 5 g filtered sample into 250-300 mL Erlenmeyer flask. Pipette 50 mL alcoholic KOH solution into flask. Connect flask with air condenser and boil until fat is completely saponified (30 min). Cool and titrate with 0.5 N HCl using phenolphathalein as indicator. Conduct blank determination along with that on sample, using same pipette 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 or oil)

Where

B = mL of 0.5 N HCl required by blank

S = mL of 0.5 N HCl required by sample

10. Water activity determination

Water activity will be, by definition, equal in all phases at equilibrium. It is defined in any mixture as the ratio of the saturated vapor pressure of water present (p_w) to that of pure water (p^o_w) at the same temperature (see equation below).

$$a_w = p_w / p^o_w$$

In this way, water activity is replaced by the equilibrium relative humidity. The fixed water activity of the reaction medium was determined by pre-equilibation of the reaction mixtures using saturated salt solution. To adjust the water activity, the immobilized enzymes or substrate solutions were equilibrated over saturated salt solutions in close vessels at room temperature for 72 hours. Salt solution with different water activity was used for this purpose (Table 31).

Table 31. Water activity of different saturated salt solutions at 25°C.

Saturated salt solution	Water activity (a_w)
LiBr	0.07
LiCl	0.11
CH_3COOK	0.25
$MgCl_2$	0.33
K_2CO_3	0.43
$Mg(NO_3)_2$	0.55
NaCl	0.75
$K_2Cr_2O_7$	0.97

Source : Humeau *et al.* (1998)

11. Determination of molecular mass of protein by SDS-PAGE

The molecular mass of sample protein was determined by comparison the R_f of sample protein with the curve plotted between R_f of standard protein against their log molecular masses (log MW) under SDS-PAGE as below. The standard proteins are myosin (201 kDa), β-galactosidase (120 kDa), bovine serum albumin (100 kDa), ovalbumin (60 kDa), carbonic anhydrase (38 kDa), Soybean trypsin inhibitor (29.7 kDa), lysozyme and (20.7 kDa).

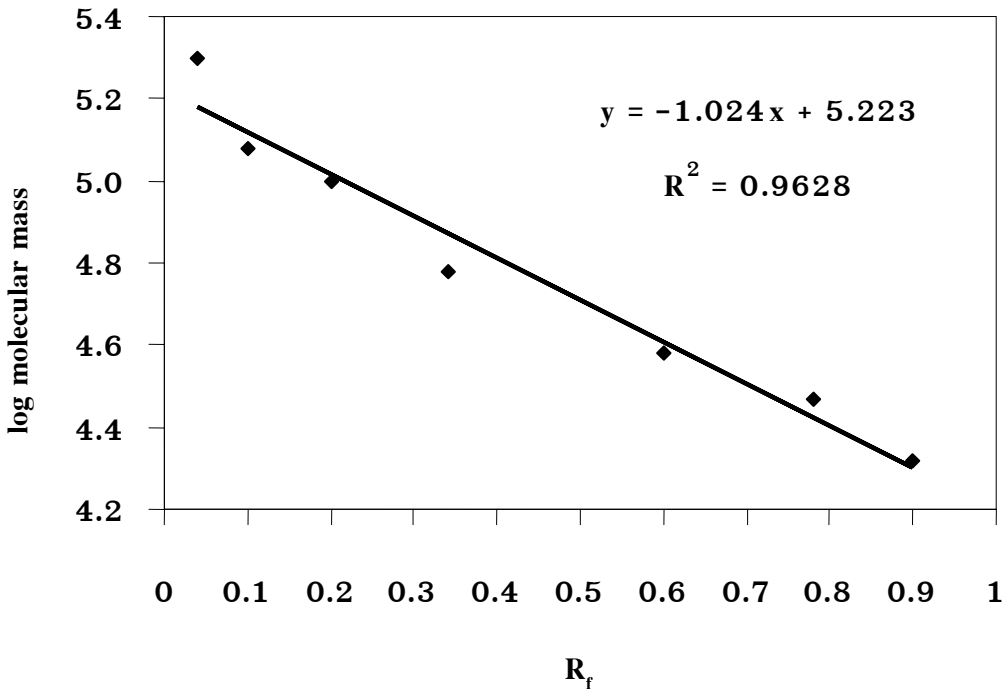
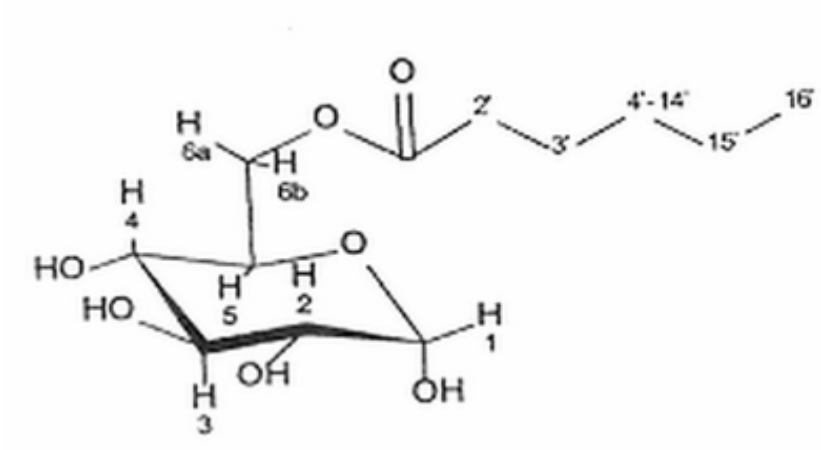


Figure 76. The curve of log molecular mass of standard protein against R_f under SDS-PAGE.

12. ^{1}H chemical shifts for 6-*O*-palmityl-α-D-glucopyranoside

Table 32. ^{1}H chemical shifts for 6-*O*-palmityl-α-D-glucopyranoside.

Proton position	C16-*O*-Glu[a] Chemical shift (ppm)	Glucose Chemical shift (ppm)
H-1	5.10	5.19
H-2	3.37	3.51
H-3	3.67	3.71
H-4	3.29	3.39
H-5	3.96	3.82
H-6a	4.35	3.82
H-6b	4.22	3.73
H-2'	3.32	
H-3'	1.61	
H-4'-H14'	1.27	
H-15'	1.30	
H-16'	0.87	



[a]The signals were relative to residual solvent signal:CHD_2OD 3.31 ppm run in $CD_3OD/CDCl_3$ (60:40) at 50°C.

13. Nucleotide sequence of 16S rDNA gene of *Streptomyces thermocarboxydus* ME168

Sample Name: ME168

Identify : *Streptomyces thermocarboxydus*

16S rDNA Sequence (1466 bp)

TACCTTGTTACGACTTCGTCCCAATCGCCAGTCCCACCTTCGACAGCTCCCTCCCCACAAGGGGGTTGGGCCACCGGCTTCG
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CGCATTTCACCGCTACACCAGGAATTCCGATCTCCCCTACCGAACTCTAGCCTGCCCGTATCGACTGCAGACCCGGGGTTAA
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TGAGCCATTACCTCACCAACAAGCTGATAGGCCGCGGGCTCATCCTGCACCGCCGGAGCTTTCGATCACCAAGGATGCCCAA
GGCGATCAGTATCCGGTATTAGACCCCGTTTCCAGGGCTTGTCCCAGAGTGCAGGGCAGATTGCCCACGTGTTACTCACCCG
TTCGCCACTAATCCACCCCGAAGGGCTTCATCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCGTC

Blast Result

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman(1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

RID: 1145973922-24206-3561665927.BLASTQ4

Database: All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS, environmental samples or phase 0, 1 or 2 HTGS sequences) 3,867,695 sequences; 17,091,186,980 total letters

Streptomyces thermocarboxydus DSM 44293 16S ribosomal RNA gene, complete sequence

Length=1500

Score = 2898 bits (1462), Expect = 0.0
Identities = 1465/1466 (99%), Gaps = 0/1466 (0%)
Strand=Plus/Minus

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14. Nucleotide sequence of 16S rDNA gene of *Burkholderia multivorans* PSU-AH130

Sample Name: PSU-AH130

Identify : *Burkholderia multivorans*

16S rDNA Sequence (1490 bp)

GTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGCAGCACGGGTGCTTGCACCTGGT
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TACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGCCTTCGG
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TCTGTTAAGACAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGTGACTGGCAGGCTAGAGTATGGCAGAGGGGG
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ACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGTTGTT
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TCGGAATCCTGAAGAGATTCGGGAGTGCTCGAAAGAGAACCGGCGCACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTG
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CAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATACAATGGTCGGAAC
AGAGGGTTGCCAACCCGCGAGGGGGAGCTAATCCCAGAAAACCGATCGTAGTCCGGATTGCACTCTGCAACTCGAGTGCATG
AAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCA
TGGGAGTGGGTTTTACCAGAAGTGGCTAGTCTAACCGTAAGGAGGACGGTCACCACGGTAGGATTCATGACTGGGGTGAAGT
CGTAACAAGGTAAC

Blast result

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

RID: 1171300927-15257-136494918845.BLASTQ1

Database: All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS, environmental samples or phase 0, 1 or 2 HTGS sequences) 4,950,527 sequences; 19,726,293,847 total letters

Query = Length=1490

Burkholderia multivorans strain Uwc 10 16S ribosomal RNA gene, partial sequence

Length=1493

Score = 2890 bits (1458), Expect = 0.0
Identities = 1482/1490 (99%), Gaps = 0/1490 (0%)
Strand=Plus/Plus

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Query  1     GTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGC  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2     GTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTACACATGCAAGTCGAACGGC  61

Query  61    AGCACGGGTGCTTGCACCTGGTGGCGAGTGGCGAACGGGTGAGTAATACATCGGAACATG  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  62    AGCACGGGTGCTTGCACCTGGTGGCGAGTGGCGAACGGGTGAGTAATACATCGGAACATG  121

Query  121   TCCTGTAGTCCCCCATAGCCCGGCGAAAGCCGGATTAATACCGCATACGATCCACGGATG  180
             ||||||||     |||||||||||||||||||||||||||||||||||| |||||||
Sbjct  122   TCCTGTAGTGGGGGATAGCCCGGCGAAAGCCGGATTAATACCGCATACGATCTACGGATG  181

Query  181   AAAGCGGGGGGACCTTCGGGCCTCGCGCTATAGGGTTGGCCGATGGCTGATTAGCTAGTTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  182   AAAGCGGGGGGACCTTCGGGCCTCGCGCTATAGGGTTGGCCGATGGCTGATTAGCTAGTTG  241

Query  241   GTGGGGTAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGACGACCAGCCAC  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  242   GTGGGGTAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGACGACCAGCCAC  301

Query  301   ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAA  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  302   ACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAA  361

Query  361   TGGGCGAAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGC  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  362   TGGGCGAAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGC  421

Query  421   ACTTTTGTCCGGAAAGAAATCCTTGGCTCTAATACAGTCGGGGGATGACGGTACCGGAAG  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  422   ACTTTTGTCCGGAAAGAAATCCTTGGCTCTAATACAGTCGGGGGATGACGGTACCGGAAG  481

Query  481   AATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTAA  540
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  482   AATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTAA  541

Query  541   TCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTCTGTTAAGACAGATGTGAAATCCCC  600
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542   TCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTCTGTTAAGACAGATGTGAAATCCCC  601

Query  601   GGGCTCAACCTGGGAACTGCATTTGTGACTGGCAGGCTAGAGTATGGCAGAGGGGGGTAG  660
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  602   GGGCTCAACCTGGGAACTGCATTTGTGACTGGCAGGCTAGAGTATGGCAGAGGGGGGTAG  661

Query  661   AATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCAG  720
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  662   AATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCAG  721

Query  721   CCCCCTGGGCCAATACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATA  780
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  722   CCCCCTGGGCCAATACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATA  781
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Query  781   CCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGTTGTTGGGGATTCATTTCCTTAGTA  840
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  782   CCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGTTGTTGGGGATTCATTTCCTTAGTA  841

Query  841   ACGTAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAA  900
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  842   ACGTAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAA  901

Query  901   GGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAA  960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  902   GGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAA  961

Query  961   AAACCTTACCTACCCTTGACATGGTCGGAATCCTGAAGAGATTCGGGAGTGCTCGAAAGA  1020
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Sbjct  962   AAACCTTACCTACCCTTGACATGGTCGGAATCCTGAAGAGATTCGGGAGTGCTCGAAAGA  1021

Query  1021  GAACCGGCGCACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA  1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1022  GAACCGGCGCACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAA  1081

Query  1081  GTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCTACGCAAGAGCACTCTAAGGAGACT  1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1082  GTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCTACGCAAGAGCACTCTAAGGAGACT  1141

Query  1141  GCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTA  1200
             |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  1142  GCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCACGGCCCTTATGGGTA  1201

Query  1201  GGGCTTCACACGTCATACAATGGTCGGAACAGAGGGTTGCCAACCCGCGAGGGGGAGCTA  1260
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1202  GGGCTTCACACGTCATACAATGGTCGGAACAGAGGGTTGCCAACCCGCGAGGGGGAGCTA  1261

Query  1261  ATCCCAGAAAACCGATCGTAGTCCGGATTGCACTCTGCAACTCGAGTGCATGAAGCTGGA  1320
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1262  ATCCCAGAAAACCGATCGTAGTCCGGATTGCACTCTGCAACTCGAGTGCATGAAGCTGGA  1321

Query  1321  ATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACAC  1380
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1322  ATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGTCTTGTACACAC  1381

Query  1381  CGCCCGTCACACCATGGGAGTGGGTTTTACCAGAAGTGGCTAGTCTAACCGTAAGGAGGA  1440
             |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  1382  CGCCCGTCACACCATGGGAGTGGGTTTTACCAGAAGTGGCTAGTCTAACCGCAAGGAGGA  1441

Query  1441  CGGTCACCACGGTAGGATTCATGACTGGGGTGAAGTCGTAACAAGGTAAC  1490
             ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1442  CGGTCACCACGGTAGGATTCATGACTGGGGTGAAGTCGTAACAAGGTAAC  1491
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