CHAPTER 3

RESULTS

3.1 Lipid and protein content determination

Table 10 Lipid and protein content of LDL subfractions for 1-DE

	Triglycerides (mg/dl)	**Total Cholesterol (mg/dl)**	**Protein (mg/ml)**
Individual 1			
sdLDL	19.7	211.4	0.43
bdLDL	11.2	52.3	0.22
Individual 2			
sdLDL	27.7	218.0	0.36
bdLDL	16.9	66.6	0.19
Individual 3			
sdLDL	23.3	156.0	0.58
bdLDL	18.5	55.5	0.25

Table 11 Lipid and protein content of LDL subfractions for 2-DE

	Triglycerides (mg/dl)	**Total Cholesterol (mg/dl)**	**Protein (mg/ml)**
Individual 1			
sdLDL	27.3	211.1	0.52
bdLDL	14.6	59.7	0.40
Individual 2			
sdLDL	9.6	85.8	0.47
bdLDL	8.4	36.1	0.45
Individual 3			
sdLDL	6.3	99.1	6.7
bdLDL	10.7	102.3	5.25
Individual 4			
sdLDL	60.6	167.5	5.11
bdLDL	76.5	56.8	1.80

3.2 One-dimensional gel electrophoresis (1-DE)

Five micrograms of proteins from fractions of sdLDL and bdLDL were separated by SDS-polyacrylamide gel electrophoresis and subsequently digested with trypsin and finally analysed by LC/ESI-ion trap MS/MS (Figure 19).

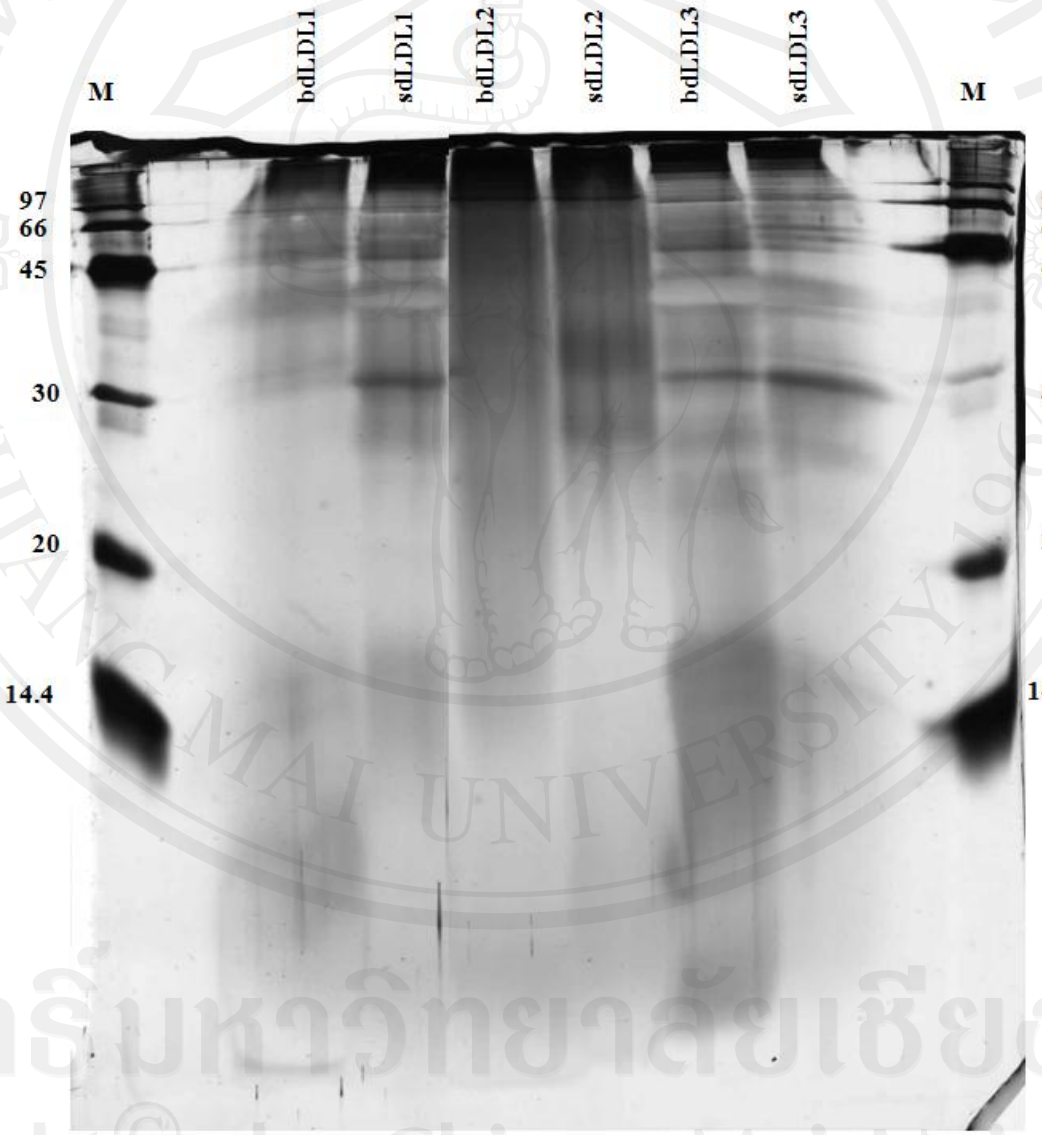


Figure 19 Protein pattern of sdLDL and bdLDL by 1-DE separation. Five micrograms of protein were separated by 1-DE followed by LC-MS/MS.

The analyzed data were subjected to the database search via Mascot software (Matrix Science, London, UK) against the NCBI database for proteins identification. Interesting proteins are shown in Table 12.

Table 12 List of interesting proteins from LDL subfractions by 1-DE separation

Protein name	**Acc. No.**	**Amino acid sequence**	**Function**
Apolipoprotein B-100	gi|225311	EEEMLENVSLVCPK	Ligand for LDL receptors (apo B/E)
Apolipoprotein E3 22kd Fragment Lys146glu Mutant	gi|15826264	LAVYQAGAR	Ligand for the LDL receptor (apo B/E) and hepatic receptor.
Apolipoprotein M	gi|55961582	SSGVTG	Plays a role in pre-β HDL formation [61]
Apolipoprotein L3	gi|13374353	FTEEATK	Involved in programmed cell death [62, 63]

Table 12 (Continued) List of interesting proteins from LDL subfractions by 1-DE separation

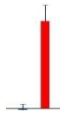
Protein name	**Acc. No.**	**Amino acid sequence**	**Function**
Apolipoprotein E2 (Apoe2, D154a Mutation)	gi|157832106	SELEEQLTPVAEETR	Ligand for the LDL receptor (apo B/E)
Apolipoprotein C-III	gi|186972736	DALSSVQESQVAQQAR	Inhibit lipoprotein lipase
Apolipoprotein B fragment	gi|1340151	FSSKYLR	Ligand for LDL receptors (apo B/E)

Comparison of protein intensities was performed using MultiExperiment Viewer (MeV) software. Student's t-test was used to determine the statistical differences with *P*-value less than 0.05. Proteins that showed differential expression between sdLDL and bdLDL are shown in Table 13. Of 11 proteins, 3 and 8 were predominantly found in sdLDL and bdLDL respectively.

Table 13 List of 11 identified proteins that showed differential expression along with their intensities from sdLDL fraction compared with bdLDL fraction.

Protein name	**Acc. No.**	**Function**	**Relative intensity**
Up-regulated in sdLDL			
Phospholipase A1	gi|*7706661*	hydrolyze phospholipids and triacylglycerol	
Teashirt homolog1	gi|*68533139*	involved in transcriptional regulation	
Zinc finger protein 407	gi|*7020321*	involved in transcriptional regulation	

Table 13 (Continued) List of 11 identified proteins that showed differential expression along with their intensities from sdLDL fraction compared with bdLDL fraction.

Protein name	Acc. No.	Function	Relative intensity
Up-regulated in bdLDL			
Lysozyme	gi|*1470345*	catalysis of the hydrolysis of the 1,4-β -linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine residues in a peptidoglycan	
Heat repeat-containing protein 5B	gi|*7243209*	unknown	

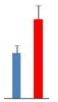
Bar graphs represent the relative intensities of each protein in sdLDL fraction (blue) and bdLDL fraction (red). Error bars are standard deviation of the averaged intensities.

Table 13 (Continued) List of 11 identified proteins that showed differential expression along with their intensities from sdLDL fraction compared with bdLDL fraction.

Protein name	Acc. No.	Function	Relative intensity
Up-regulated in bdLDL			
p-53 associated protein	gi|*1079710*	inactivate the transcriptional activity of p53	
ATP-dependent helicase 1	gi|*8977885*	unwinding of a DNA helix	
G-protein coupled receptor 75	gi|*5803025*	transduce extracellular signals across the cell membrane	

Bar graphs represent the relative intensities of each protein in sdLDL fraction (blue) and bdLDL fraction (red). Error bars are standard deviation of the averaged intensities.

Table 13 (Continued) List of 11 identified proteins that showed differential expression along with their intensities from sdLDL fraction compared with bdLDL fraction.

Protein name	Acc. No.	Function	Relative intensity
Up-regulated in bdLDL			
A disintegrin and metalloproteinase with thrombospondin motifs 15	gi|*21265058*	digest extracellular matrix	
actin-related protein 3B-like	gi|*239749318*	regulate the actin filament polymerization	
Peptidyl-glycine alpha-amidating monooxygenase	gi|*293336314*	catalyze amidation reaction	

Bar graphs represent the relative intensities of each protein in sdLDL fraction (blue) and bdLDL fraction (red). Error bars are standard deviation of the averaged intensities.

3.3 Two-dimensional gel electrophoresis (2-DE)

Three hundred micrograms of proteins from sdLDL fraction were separated by two-dimensional gel electrophoresis, subsequently digested with trypsin and finally analyzed by LC/ESI-ion trap MS/MS. There were 10 interesting spots. Apolipoprotein A-I, E, C-III, B and protein phosphatase 2a were identified in sdLDL (Figure 20).

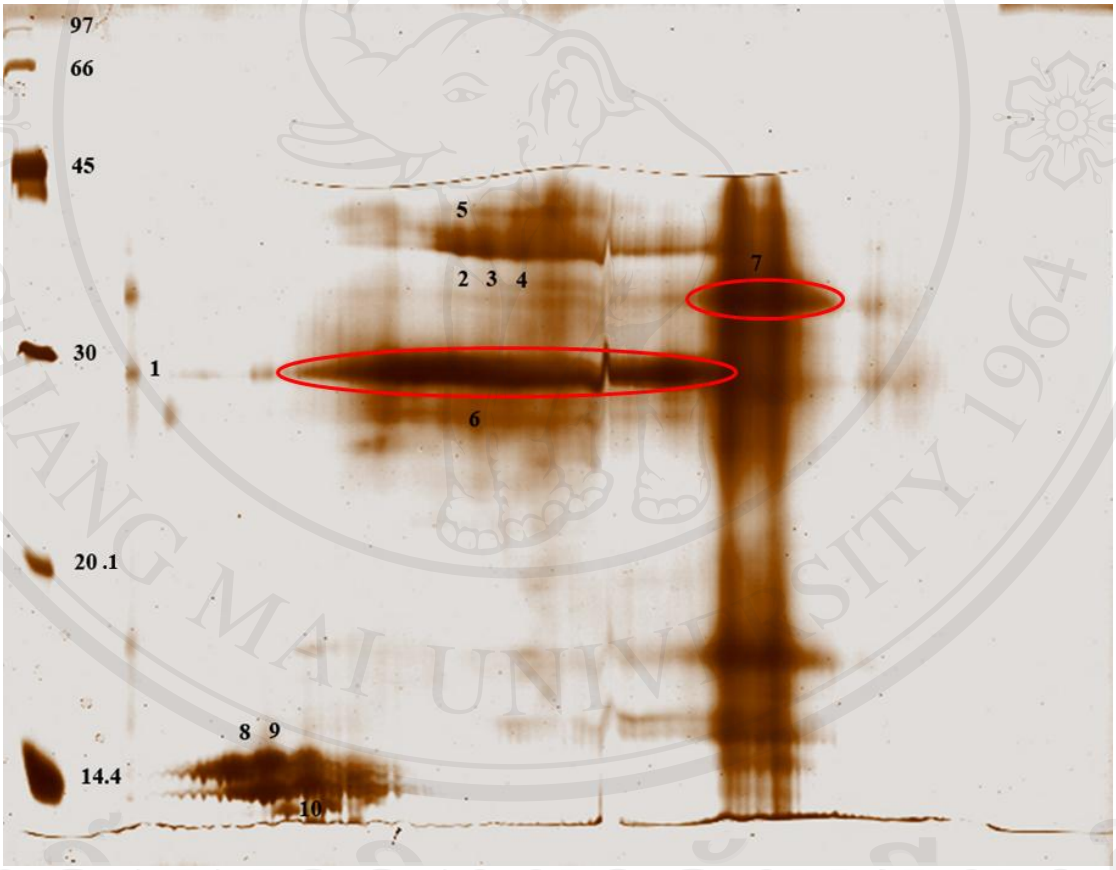


Figure 20 Protein pattern of sdLDL by 2-DE separation.

Table 14 Protein identification of sdLDL from 2-DE

Spot No.	Protein	Acc. No.	Exp. MW(Da)/pI	Theo. MW(Da)/pI	Score
1	Apolipoprotein A-I	gi|229479	29100/3.19	28329/5.27	64
2	Apolipoprotein E	gi|178849	38882/5.76	36302/5.65	235
3	Apolipoprotein E	gi|178849	38487/5.98	36302/5.65	172
4	Apolipoprotein E	gi|178849	38289/6.27	36302/5.65	152
5	Protein Phosphatase 2A (PP2A)	gi|122921195	40263/5.76	45927/6.27	50
6	Apolipoprotein A-I	gi|178775	29438/6.19	28944/5.45	96
7	Apolipoprotein B	gi|62630102	35131/8.25	442746/7.06	137
8	Apolipoprotein C-III	gi|521205	16343/3.78	10815/5.23	55
9	Apolipoprotein C-III	gi|521205	16343/4.17	10815/5.23	54
10	Apolipoprotein C-III	gi|521205	14011/4.56	10815/5.23	108

Similar procedures were also performed in bdLDL. There were 5 interesting spots found in bdLDL including apolipoprotein E, C-III and D (Figure 21).

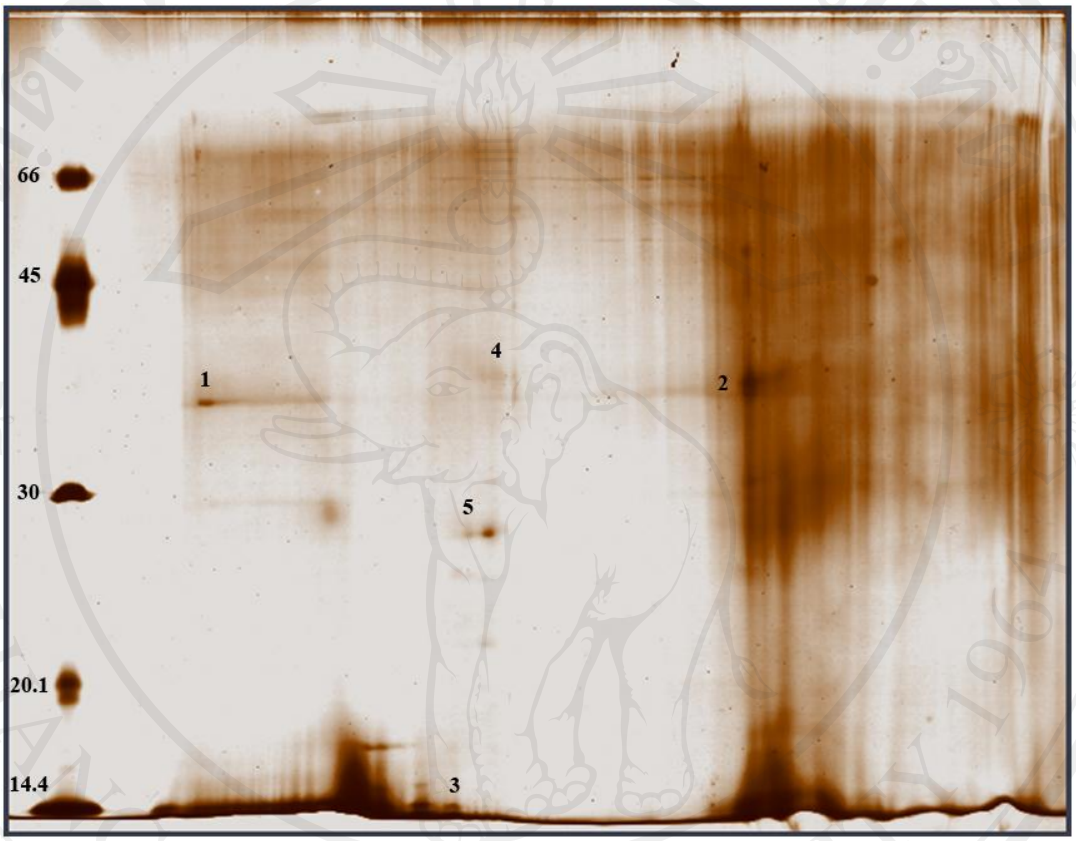


Figure 21 Protein pattern of bdLDL by 2-DE separation.

Table 15 Protein identification of bdLDL from 2-DE

Spot No.	Protein	Acc. No.	Exp. MW(Da)/pI	Theo. MW(Da)/pI	Score
1	Apolipoprotein E	gi|178849	36818/3.12	36302/5.65	126
2	Apolipoprotein E	gi|178849	37500/7.98	36302/5.65	389
3	Apolipoprotein C-III	gi|521205	14400/5.14	10815/5.23	68
4	Apolipoprotein E	gi|178849	38352/5.49	36302/5.65	142
5	Apolipoprotein D	gi|4502163	27827/5.33	21547/5.06	24*

*not significant score