TABLE OF CONTENT

ABSTRACT	ii
ABSTRAK	iv
AKNOWLEDGMENT	vi
ABBREVIATION	viii
TABLE OF CONTENTS	xi
LIST OF TABLES	xviii
LIST OF FIGURES	XX

CHAPTER 1: Introduction	1
CHAPTER 2: Literature Review	4
2.1 Importance of milk in human's nutrition	4
2.2 Fermented milk products	5
2.2.1 Milk fermentation and biomedical changes	6
2.2.2 Probiotics	9
2.2.3 Health benefits of probiotics	11
2.2.4 Lactic acid bacteria	11
2.2.5 Yoghurt culture bacteria	12
2.3 Yoghurt	13
2.3.1Health benefits of yoghurt	14
2.3.2 Syneresis	16
2.3.3 Yoghurt appearance	17
2.4 Milk proteins	
2.4.1 Changes in milk protein structure during fermentation	19
2.4.2 Proteolysis in fermented milk	22
2.5 Renin-Angiotensin System	22

2.5.1 Angiotensin Converting Enzyme	22
2.5.2 ACE-Inhibition	23
2.5.3 Bioactive peptide with ACE- inhibiting properties	24
2.6 Herbs and Benefits	26
2.6.1 Angelica sinensis	27
2.6.1.1 Description	27
2.6.1.2 Function and benefits	27
2.6.2 Codonopsis pilosula	29
2.6.2.1 Phytochemicals	29
2.6.2.2 Function and benefits	30
2.6.3 Illicium verum	31
2.6.3.1 Phytochemicals	31
2.6.3.2 Health and benefits	32
2.6.4 Lycium barbarum	34
2.6.4.1 Phytochemicals	35
2.6.4.2 Function and benefits	35
2.6.5 Momordica grosvenori	37
2.6.6 Psidium guajava	39
2.6.6.1 Phytochemicals	40
2.6.6.2 Medical properties	.40
CHAPTER 3: Methodology	42
3.1 Materials	42
3.1.1 Milk	42
3.1.2 Starter culture	. 42
3.1.3 Herbs	42
3.1.4 Chemicals	42

3.2 Methods	43
3.2.1 Preparation of herb powder	43
3.2.2 Preparation of herbal extract	.43
3.2.3 Yoghurt manufacturing	44
3.2.4 Preparation of herbal yoghurt	.45
3.3 Physicochemical analysis	.45
3.3.1 Sampling	45
3.3.2 pH evaluation	46
3.3.2.1 pH changes during fermentation	46
3.3.3 Total Titratable acidity (TTA)	47
3.3.4 Moisture content	47
3.3.5 Sensory evaluation	48
3.4 Microbiology analysis	48
3.4.1 Preparation of dilutions	48
3.4.2 Enumeration of Streptococcus thermophilus	49
3.4.2.1 Preparation of culture media	49
3.4.2.2 Inoculation and enumeration	49
3.4.3 Enumeration of <i>Lactobacillus spp</i>	. 49
3.4.3.1 Media preparation	49
3.4.3.2 Inoculation and enumeration of bacteria	50
3.4.4 Viable cell count calculation	50
3.5 Determination of peptides and free amino acids formation	
from milk protein proteolysis	51
3.5.1 Cd-ninhydrin method	51
3.5.2 Sodium dodecyl sulphate-polyacrylamide gel	
electrophoresis (SDS-PAGE)	52

3.5.2.1 SDS- PAGE solutions	52
3.5.2.2 Casein extraction	53
3.5.2.3 Preparation of SDS-polyacrylamide gels	54
3.5.3 Yoghurt water extraction for OPA and ACE experiments	58
3.5.4 The OPA test	58
3.5.4.1 Preparation of OPA reagent	
3.5.4.2 Determination of peptide concentration	59
3.5.4.3 Preparation of standard curve	59
3.5.5 The ACE inhibition activity of herbal yoghurt	59
3.5.5.1 Substrates and chemicals	59
3.5.5.2 Preparation of rabbit lung extract	60
3.5.5.3 ACE activity assay	60
3.5.5.4 ACE inhibition assay	60
3.5.5.5 Inhibition of ACE by herb extract	61
3.6. Statistical analysis	62
CHAPTER 4: RESULTS	63
4.1 Chemical and microbial analysis of yoghurts with different stages	
of fermentation	63
4.1.1 Changes of pH in fermentation of milk in the presence	
of herbs' water extract at 41°C	63
4.1.2 Changes of TTA during yoghurt fermentation in the presence	
of herbal water extract	64
4.1.3 Changes in pH and TTA of yoghurts with initial pH 4.5 during	
storage at 4°C	65
4.1.4. Enumeration of <i>Lactobacillus spp</i> . during storage in MRS Agar	67
4.1.5. Viability of <i>Streptococcus thermophilus</i> in yoghurts in M17 Agar	69

4.2. Acidity of yoghurts during refrigerated storage (4°C)	71
4.2.1 Changes in pH of yoghurts	71
4.2.2 Changes of total titratable acidity of yoghurts	72
4.3 Viable cells count in yoghurts during refrigerated storage (4°C)	73
4.3.1 Changes in viable cells count of <i>Lactobacillus spp</i>	73
4.3.2 Changes in viable cells count of <i>Streptococcus thermophilus</i>	74
4.4 Proteolysis of yoghurts during refrigerated storage	76
4.4.1 Effects on peptide concentration	76
4.4.2 Effects on total free amino acids	78
4.4.3 Effects on Angiotensin-1 converting enzyme (ACE) activity	81
4.4.4 ACE-Inhibitory effects of yoghurts	81
4.5 SDS-PAGE, gel electrophoretic patterns of yoghurts during	
fermentation and storage	86
4.6 Average of moisture content changes in yoghurts during storage	95
4.7 Sensory changes of herbal-yoghurts and plain-yoghurt	95
CHAPTER 5: DISCUSSION	
5.1 Acidification of herbal yoghurt	
5.1.1 Acidification of yoghurt during fermentation and storage	99
5.2 Microbial population in yoghurts: Effect of herbs on viable	
S.thermophilus and Lactobacillus spp. in yoghurt during storage	
5.3 Changes in proteolytic activity	
5.3.1 Herbs and its effect on peptide concentration of yoghurt	
5.3.2 Effects of herbs on total free amino acids during storage	
in yoghurts	
5.3.3 ACE inhibitory activities of herbal-yoghurts	

5.3.4 SDS-PAGE, gel electrophoresis of yoghurts during	
fermentation and storage	112
5.4 Organoleptic properties of herbal-yoghurt	
5.5 CONCLUSION	115
REFERENCES	
APPENDIXES	140

APPENDIXES

- Appendix 1: Time interval specifications for sampling
- Appendix 2: Table 4.1: Changes of pH during fermentation of yoghurts at 41°C to final pH 4.5
- Appendix 3: Table 4.2: Changes of TTA in yoghurts during fermentation at 41°C with final pH 4.5
- Appendix 4: Table 4.3 Changes of pH in yoghurts with initial pH 4.5 during storage
- Appendix 5: Table 4.4 Changes of TTA in yoghurt with initial pH 4.5 during storage
- Appendix 6: Table 4.7 Changes of pH and %TTA in yogurts (18 hour fermentation) in presence of herbs during storage at 4°C.
- Appendix 7: Table 4.12 Effect of herbs water extract on ACE activity and ACE inhibition
- Appendix 8: Table 5.1 Correlations within pH, TTA, *Lactobacillus sp.* CFU/ml ×10⁶, *S.thermophilus* CFU/mL×10⁸
- Appendix 9: Table 5.2 Correlation of specific activity of yoghurts with IC 50 and OPA
- **Appendix 10: Changes of moisture content in yogurt during storage**
- Appendix 11: Different concentration (mg/ml) of tryptone and average of tryptone absorbance at 340 nm wavelength
- **Appendix 12: Typical standard curve of tryptone (mg/ml)**
- Appendix 13: Typical leucin standard curve with different concentration (mM/ml) of Leucin
- Appendix 14: Changes of overall aroma in yoghurts during storage
- Appendix 15: Changes of overall appearance of yogurts during storage
- Appendix 16: Changes of overall taste in yogurts during storage

Appendix 17: Changes of sourness in yoghurts during storage

Appendix 18: Changes of bitterness of yogurts during storage

LIST OF TABLES

Table 2.1 Nutrients of various milks	5
Table 2.2 Comparison of nutrient composition in various milk products	7
Table 2.3 The milk proteins, their isoionic points and molecular weights	21
Table 2.4 Bioactive peptides identified from milk products	25
Table 3.1 Concentration of herbs in specified volume	44
Table 3.2 Separating gel mix for total volume of 20ml	54
Table 3.3 Stacking gel mix	55
Table 4.5 Enumeration of <i>Lactobacillus sp.</i> CFU ×10 ⁵ /mL in yogurts during storage at 4°C	68
Table 4.6 Enumeration of <i>S. thermophilus</i> (CFU×10 ⁸ /mL) in yoghurts stored (4°C) up to 21 days	70
Table 4.8. Enumeration of <i>Lactobacillus sp.</i> (CFU×10 ⁶ /mL) in yoghurts during storage	74
Table 4.9 Enumeration of S. thermophilus of yoghurts during storage	75
Table 4.10 Changes of peptide concentration (mg/ml) of yoghurts during storage	77
Table 4.11 Changes of total free amino acids(mM of leucine eq/ml yoghurt) in yoghurts during storage	78
Table 4.13 Percentage of ACE- Inhibition in herbal andplain yogurts within two weeks storage	82
Table 4.14 Changes of specific activity (unit/min/mg peptides) in yoghurt during storage	84
Table 4.15 IC ₅₀ % of yoghurts during 4 weeks storage at 4°C	85

Tabel 4.16 Comparision of densitometric values of SDS-PAGE protein	
in A.sinensis-yogurt with respective plain-yoghurt during	
storage at 4 °C	88
Table 4.17 Comparision of densitometric values of SDS-PAGE proteins	
in <i>C.pilosula</i> -yogurt with respective plain-yoghurt during	
storage at 4 °C	89
Table 4.18 Comparision of densitometric values of SDS-PAGE protein	
in I. verum-yogurt with respective plain-yoghurt during	
storage at 4 °C	90
Table 4.19. Comparision of densitometric values of SDS-PAGE protein	
in L.barbarum-yogurt with respective plain-yoghurt during	
storage at 4 °C	91
Table 4.20 Comparision of densitometric values of SDS-PAGE protein	
in <i>M.grosvenori</i> -yogurt with respective plain-yoghurt during	
storage at 4 °C	92
Table 4.21 Comparision of densitometric values of SDS-PAGE protein	
in P.guajava-yogurt with respective plain-yoghurt during	
storage at 4 °C	93

LIST OF FIGURES

Figure 2.1 Lactic acid bacteria	13
Figure 2.2 Interaction of casein micelles with whey protein after heating	20
Figure 2.3 The renin- angiotensin system	22
Figure 2.5. Angelica sinensis root	27
Figure 2.6 Codonopsis pilosula dried root	29
Figure2.6 Illicium verum	31
Figure 2.7 Lycium barbarum	34
Figure 2.8 Momordica grosvenri	37
Figure 2.9 Psidium guajava	39
Figure 4.1 Changes of pH during the fermentation of milk in the presence of different herbs at 41°C	64
Figure 4.2 Changes of TTA during fermentation of milk in the presence of different herbs at 41°C	65
Figure 4.3 Changes in pH of yoghurts during 21 days storage at 4°C	66
Figure 4.4 Changes in TTA of yoghurts during storage at 4°C	67
Figure 4.5 Viable count of <i>Lactobacillus sp.</i> in yoghurts during storage at 4°C	68
Figure 4.6 Viable count of <i>Streptococcus. thermophilus</i> (CFU×10 ⁸ /mL) in yoghurt during storage	70
Figure 4.7 Changes of pH in yoghurts during storage	71

Figure 4.8 Changes in total titrable acidity (TTA) of yoghurts during Storage	72
Figure 4.10 Changes of <i>Lactobacillus sp.</i> count (CFU ×10 ⁶ /ml) of yoghurts during storage	74
Figure 4.11 Changes of <i>Streptococcus thermophilus</i> (CFU ×10 ⁸ /ml) in voghurts during storage at 4°C	76
Figure 4.11 Changes of nontide concentration (mg/ml) in we shouts	
Figure 4.11 Changes of peptide concentration (mg/mi) in yognurts	//
Figure 4.12 Total free amino acid (mM of leucine eq/ml yoghurt) of yoghurts during storage at 4°C	79
Figure 4.13 ACE activity of herb extract	80
Figure 4.14 Percentage of ACE-inhibition of herb extract	81
Figure 4.15 percentage of inhibition in yoghurts during storage at 4°C	82
Figure 4.16 Changes of IC_{50} (mg/g) of yoghurts against storage days	86
Figure 4.17 Changes of protein from SDS-PAGE analysis <i>A.sinensis</i> -yoghurts and control during storage at 4 °C	88
Figure 4.18 Changes of protein from SDS-PAGE analysis <i>C.piloula</i> -yoghurts and control during storage at 4 °C	89
Figure 4.19 Changes of protein from SDS-PAGE analysis <i>I.verum</i> -yoghurts and control during storage at 4 °C	90
Figure 4.20 Changes of protein from SDS-PAGE analysis <i>L.barbarum</i> -yoghurts and control during storage at 4 °C	91
Figure 4.21 Changes of protein from SDS-PAGE analysis <i>M.grosvenori</i> - yoghurts and control during storage at 4 °C	92

Figure 4.22 Changes of protein from SDS-PAGE analysis P.guajava-	
yoghurts and control during storage at 4 °C	93
Figure 4.23 SDS–PAGE patterns of proteins in A.sinensis-yogurt	
and plain-yoghurt	94
Figure 4.24 SDS–PAGE patterns of proteins in <i>I.verums</i> -yogurt	
and plain-yoghurt	94
Figure 4.25 Changes of overall aroma in herbal-yoghurts and	
control during storage at 4 °C	96
Figure 4.26 Changes of overall appearance in herbal-yoghurts and control	
during storage at 4 °C	96
Figure 4.27 Changes of overall taste in herbal-yognurts and control	0.
during storage at 4 °C	97
Figure 4.28 Changes of sourness in herbal-yoghurts and control	
during storage at 4 °C	97
Figure 4.29 Changes of bitterness in herbal-yoghurts and control	
during storage at 4 °C	98