View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Massey Research Online

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Research Form RF 5

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

APPLICATION FOR APPROVAL TO EMBARGO A THESIS

Pursuant to AC 98/168 (Revised 2).

Approved by Academic Board 16/02/99

Student ID number: 03230120

First name: Noriza

Surname: Ahmad

Programme of study:

Thesis title: Beef hydrolysis using Zyactinase enzyme

Chief supervisor: Assc. Prof Dr. Marie Wong

Phone extension:

As author of the above-named thesis, I request that my thesis be embargoed from public access until:

Date: 17 10 2018

for the following reason(s):

- [x] The thesis contains commercially-sensitive information
- [ ] The thesis contains information which is personal or private and/or which was given on the basis that it would not be disclosed
- [ ] Immediate disclosure of the thesis' contents would not allow the author a reasonable opportunity to publish all or part of the thesis
- [ ] Other. Please specify:

NOTE: The standard embargo is for a maximum of two years

JUSTIFICATION

The study was funded and use a commercial enzyme (Zyactinase) which develop by the company.

Vital Food who funded the research and supplied the enzyme would still like the thesis to be embargoed for 2 years.

Student's signature: Noriza Ahmad

Date: 17 10 16

Chief supervisor's signature: M. Wong

Date: 18 10 2016

☑ Approved ◯ Not Approved

Signature of representative of the Vice Chancellor: [signature]

Date: 26 01 2017

THE ENGINE OF THE NEW NEW ZEALAND

BEEF HYDROLYSIS BY ZYACTINASE^{TM} ENZYMES

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

at Massey University, Auckland,

New Zealand.

NORIZA BINTI AHMAD

2016

Abstract

Protein hydrolysis is the term that applies to all possible ways of splitting proteins to produce products with lower molecular weight. There is a continuous search for novel products derived from waste materials. In the developed nations considerable amount of meat off-cuts are discarded each year. Utilizing these leftovers by developing new technology for protein recovery and modification and production of a broad spectrum of food ingredients greatly enhances its final value.

The aim of this research was to partially hydrolyse beef meat protein with a commercial kiwifruit product called Zyactinase™, which is essentially freeze-dried kiwifruit to determine the effect of various processing conditions that influence the extent of beef meat hydrolysis. Secondly to determine the peptide and amino acid profile of the beef meat sample after hydrolysis. Thirdly to determine the relative reaction of Zyactinase™ on various beef meat protein fractions. This study also aimed to evaluate the rate and the extent of partial enzymic hydrolysis of lean beef using Zyactinase™ enzymes in order to obtain a better understanding of protein hydrolysis reaction.

Lean beef minced was partially hydrolysed using the Zyactinase enzymes for different processing times (up to 360 minutes), temperatures (27°C to 70°C) and varying enzyme concentrations. No pH adjustment on the raw material was carried out except for pH studies. The hydrolysates were collected and analysed for total nitrogen content and degree of hydrolysis. The method used to characterize the extent of protein hydrolysis was SN-TCA index (fraction of nitrogen soluble in trichloroacetic acid) also called non-protein nitrogen NPN. Peptide and amino acid in protein hydrolysates were analysed by HPLC and different protein fractions in the hydrolysates were characterised by SDS-PAGE.

The relationship between the reaction temperature, enzyme concentration and processing time to the total nitrogen and NPN were determined. The total nitrogen content remained relatively constant throughout the hydrolysis process. In addition, the NPN content increased as the temperature, processing time and enzyme concentration increased. The optimum pH range for the enzyme's activity was 4 – 5.6 and optimum temperature was 60°C. Furthermore, most of the higher molecular weight protein bands on SDS- PAGE disappeared after hydrolysis and lower molecular weight protein

bands increased in intensity. Zyactinase was also found to digest protein in the myobrilla and sarcoplasmic meat fractions at similar rates as whole beef meat.

The results provide basic understanding of the kiwifruit enzymes action toward protein that may lead to improved methods for recovering meat protein or developing new food materials.

Acknowledgements

I would like thank all who in one way or another contributed in the completion of this thesis. Firstly, I give thanks to God for protection and ability to finally get it done.

My special and heartily thanks to my supervisor, Associate Professor Marie Wong who encouraged and directed me. Her continuous support and believe that brought this work towards a completion. I am really thankful for her patience, motivation, enthusiasm, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my PhD study. For any faults I will take full responsibility. I am also deeply thankful to my co – supervisors Professor Ray Winger and Dr. Sung Je Lee with their help and guidance and insightful comment that I am able to complete this thesis.

Besides my supervisors I would like to thank Helen for her technical assistance to my project.

I am thankful to my colleagues Nayawit, Yan, Nabiha, Azie, Kataraina and Vivian for the help and support during my stay in Albany, New Zealand.

I would also like to acknowledge the support of University of Kuala Lumpur and MARA who supported me financially and gave me time to do this PhD.

Finally, I take this opportunity to express the profound gratitude from my deep heart to my beloved parents, and my siblings for their love and continuous support – both spiritually and materially.

Table of Contents

Abstract iii

Acknowledgements v

Table of Contents vi

List of Figures xiii

List of Tables xviii

Chapter 1 1

Introduction 1

Chapter 2 4

Literature Review 4

2.1 Muscle structure 4

2.2 Muscle proteins 6

2.2.1 Myofibrillar Proteins 7

2.2.1.1 Myosin 9

2.2.1.2 Actin 11

2.2.1.3 Tropomyosin 12

2.2.1.4 Troponin 12

2.2.1.5 Actinin 12

2.2.1.6 Filament protein 13

2.2.1.7 Minor regulatory proteins 13

2.2.2 Sarcoplasmic proteins 14

2.2.2.1 Myoglobin 14

2.2.2.2 Haemoglobin 15

2.2.3 Connective tissue proteins 16

2.2.3.1 Collagen 16

2.2.3.2 Elastin 18

2.3 Amino acids 18

2.4 Protein functional properties 20
2.4.1 Protein solubility 20
2.4.2 Effect of pH on protein solubility 21
2.4.3 Effect of ionic strength on protein solubility 22
2.4.4 Effect of temperature on solubility 23
2.4.5 Emulsification capacity (EC) 23
2.4.6 Viscosity 24
2.5 Protein modification via hydrolysis 24
2.6 Enzymatic modification of protein 25
2.6.1 The extent of enzymatic hydrolysis process 26
2.7 Protein hydrolysis mechanism 27
2.7.1 Degree of hydrolysis (DH) 29
2.7.2 Nitrogen to protein conversion 30
2.7.3 Bitter Peptides 31
2.8 Previous studies on meat hydrolysis 32
2.8.1 Previous studies with kiwifruit and meat hydrolysis 34
2.8.2 Proteolytic enzymes (Actinidin) 35
2.8.3 The structure of actinidin 35
2.8.4 Actinidin properties 36
2.8.5 Actinidin activity 37
2.9 Kiwifruit enzymes and the effect on food proteins 38
2.10 Collagenase properties in kiwifruit enzymes 39
2.11 Conclusions 40
Chapter 3 42
Materials and Methods 42
3.1 Chemical preparations 42
3.1.1 Acrylamide/Bis solution for SDS PAGE 42

3.1.2 Ammonium persulfate (APS) Solution (10% w/v) for SDS PAGE 42
3.1.3 Boric acid solution, 4 % w/v Kjeldahl analysis 42
3.1.4 Bradford reagent 43
3.1.5 Destaining Solution for SDS PAGE 43
3.1.6 Electrophoresis Running Buffer, pH 8.3 for SDS PAGE 43
3.1.7 Iodoacetate–KCl solution 43
3.1.8 Kjeldahl Catalyst Tablets 44
3.1.9 OPA reagent 44
3.1.10 Phosphate Buffer (0.03 M) for SDS PAGE 44
3.1.11 Phenolphthalein Indicator Solution 44
3.1.12 Derivatization reagent for SDS PAGE 45
3.1.13 Protein Electrophoresis Size Standard for SDS PAGE 45
3.1.14 Phosphate Buffer (0.1 M) for SDS PAGE 45
3.1.15 Resolving Buffer (1.5 tris-HCl, pH 8.8) for SDS PAGE 45
3.1.16 Sodium Hydroxide (NaOH), 40% w/v for Kjeldhal analysis 45
3.1.17 Sample Buffer (0.0625 M tris-HCl, pH 6.8) for SDS PAGE 45
3.1.18 Sodium dodecyl sulphate (SDS), 10 % w/v for SDS PAGE 46
3.1.19 Stacking Gel (0.5 M tris- HCl, pH 6.8) for SDS PAGE 46
3.1.20 Trifluoroacetic acid (TFA) (0.1 % v/v) for HPLC analysis 46
3.1.21 Trichloroacetic Acid (TCA) Solution (15% w/v) for precipitation of protein 46
3.2 Raw materials 47
3.2.1 Minced lean beef 47
3.2.2 Sarcoplasmic protein (water soluble protein) 48
3.2.3 Myofibrilla protein (salt-soluble protein) 49
3.2.4 Connective tissue 49
3.3 Enzymes used for hydrolysis reactions 50
3.4 Beef hydrolysis process 50

3.4.1 Hydrolysis process setup....50
3.5 General chemical analyses....56
3.5.1 Moisture content....56
3.5.2 Ash content....56
3.5.3 Fat content....57
3.5.4 pH measurement....58
3.5.4.1 pH measurement of minced beef and meat hydrolysate samples....58
3.5.4.2 pH measurement during the hydrolysis process....58
3.5.5 Total nitrogen content....59
3.5.6 Non-protein nitrogen (NPN) content....59
3.5.7 Protein determination by Bradford method....60
3.6 The effect of reaction parameters on minced beef hydrolysis....61
3.6.1 Effect of pH on the extent of hydrolysis....61
3.6.2 Effect of substrate concentration on the extent of hydrolysis....61
3.6.3 Effect of reaction time on the extent of hydrolysis....62
3.6.4 Effect of temperature on the extent of the hydrolysis....62
3.6.5 Effect of enzyme concentration on the extent of hydrolysis....62
3.7 Polyacrylamide gel electrophoresis (SDS- PAGE) analysis....63
3.7.1 Gel preparation....63
3.7.2 Sample preparation for SDS-PAGE....63
3.7.3 Run conditions for SDS-PAGE....63
3.7.4 Staining and destaining for SDS-PAGE....64
3.7.5 SDS-PAGE imaging....64
3.8 Hydrolysis of meat protein fractions (sarcoplasmic, myofibrilla and connective tissue)....64
3.9 Peptide analysis on hydrolysates....65
3.9.1 Sample preparation....65
3.9.2 Chromatographic conditions for peptide analysis....65

3.9.3 Chromatograph analysis 66
3.10 Amino acid analysis on hydrolysate 67
3.10.1 Sample preparation 67
3.10.2 Sample derivatization for amino acids 67
3.10.3 Chromatographic conditions for amino acid analysis 67
3.10.4 Chromatograph analysis. 68
3.11 Kinetic studies of meat hydrolysis 68
3.11.1 OPA analysis to determine the α- amino acid and degree of hydrolysis (DH) 69
3.11.2 Determination of initial velocity 70
3.11.3 Determination of K_m and V_{max} 70
3.12 Data analysis 71
Chapter 4 72
Effect of hydrolysis conditions on the total nitrogen and non-protein nitrogen contents 72
4.1 Characterisation of ZyactinaseTM enzyme complex 72
4.2 Proximate analysis of meat 74
4.3 Proximate analysis of hydrolysate 75
4.3.1 Hydrolysis process. 75
4.3.2 Proximate composition of hydrolysate 76
4.3.3 Moisture loss during the hydrolysis process 76
4.3.4 Changes in pH during hydrolysis process 78
4.4 Effect of hydrolysis conditions on the extent of the hydrolysis process. 82
4.4.1 The rate of hydrolysis 82
4.4.2 Effect of pH on the hydrolysis at 50°C 85
4.4.3 Effect of temperature on the hydrolysis process 90
4.4.3.1 Impact of temperature on NPN 90
4.4.3.2 Impact of temperature on SDS-PAGE results 92
4.4.4 Enzyme concentration and reaction parameters 96

4.4.5 Effect of substrate concentration.. 99
4.5 Summary 100
Chapter 5... 101
Peptide and Amino Acid Analysis of Hydrolysates 101
5.1 Peptide analysis 101
5.2 Free amino acid formation from Zyactinase™ hydrolysis of meat 105
5.3 Summary 106
Chapter 6... 107
Hydrolysis of different meat protein fractions 107
6.1 Protein separation 107
6.1.2 Hydrolysis of meat protein fractions... 107
6.2 Composition of various hydrolysis solutions for each meat fraction 108
6.3 Whole meat and meat protein fractions: extent of hydrolysis and NPN released. 109
6.4 SDS-PAGE analysis of protein fractions after hydrolysis............................. 110
6.4.1 Whole meat hydrolysis.. 110
6.4.2 Myofibrillar proteins hydrolysis 111
6.4.3 Sarcoplasmic proteins fraction 114
6.4.4 Connective tissue fraction.. 114
6.5 Enzyme hydrolysis of different protein fractions 115
6.6 Summary 117
Chapter 7... 119
Reaction kinetic of meat hydrolysis using Zyactinase™ 119
7.1 Correlation between the non-protein nitrogen concentrations (NPN) with degree of hydrolysis (DH) to determine the extent of hydrolysis. 119
7.2 Meat hydrolysis progress curve 120
7.3 Study of reaction kinetic.. 123
7.4 The effect of addition of new substrate and enzyme during hydrolysis........ 123

7. 5 Reaction rate equation 126
7.5.1 Initial velocity (V_0) for protein hydrolysis 127
7.5.2 Fitting data to classical Michaelis-Menten Kinetics 132
7.6 Enzyme Concentration 137
7.7 Summary 138
Chapter 8 139
Overall Discussion 139
Chapter 9 142
Conclusion and Recommendations 142
9.1 Conclusions 142
9.2 Recommendations for future work 143
References 144
Appendix A. 1.1 157
Appendix A. 1.2 159

List of Figures

Figure 2.1: Diagrammatic representation of striated muscle structure (Winger, 1979)... 5
Figure 2.2: Portion of two myofibrils and a sarcomere (Forrest et al., 1975).................. 8
Figure 3.1: (A) Lean beef meat, (B) Visible connective tissue and fat remove from the beef meat... 47
Figure 3.2: (A) Dimock meat grinder; (B) Mixed minced beef going through the meat grinder for the second time. 48
Figure 3.3: Grinder plate with hole size of 3 mm attached to meat grinder. 48
Figure 3.4: Experimental set up for hydrolysis process 52
Figure 3.5: Gate stirrer used to mix beef samples during hydrolysis experiments. 52
Figure 3.6: Qualitative observations during hydrolysis of beef: Hydrolysis conditions; 60°C, 2% w/w enzyme concentration. 55
Figure 4.1: SDS-PAGE of the enzyme ZyactinaseTM on 12% gel; Lane Z: 1.0 g protein /ml buffer and Lane M: Molecular weight marker. (a) Actinidin, (b) kiwellin, (c) TLP and (d) KiTH 73
Figure 4.3: Total nitrogen content in hydrolysate reactions at 40°C and 2% enzyme concentration: (A) wet weight basis, (B) dry weight basis. Mean value ± standard deviation, for n=4-6 trials.. 80
Figure 4.4: Changes of pH during hydrolysis at hydrolysis process at 40°C and 2% enzyme concentration.. 81
Figure 4.5: NPN content (g/100 g dry sample) of hydrolysates at 60°C without enzyme addition (control ●) and 2% enzyme concentration (○). Data points are mean values ± standard deviation, n = 3.. 82
Figure 4.6: Total nitrogen content for hydrolysate at 40°C (A) without enzyme addition (B) 1% enzyme concentration (C) 4% enzyme concentration. Data points are mean values ± standard deviation, n = 2 to 6... 84
Figure 4.7: Changes in pH value during the hydrolysis process at 50°C and 2% enzyme concentration. Data points are mean values ± standard deviation n = 3...................... 86
Figure 4.8: The change in pH after addition of enzyme in the mixture (pH was measured at 90 seconds reaction time). Data points are mean values ± standard deviation n = 3 86
Figure 4.9: NPN content of the hydrolysates for different initial pHs of diluted meat, at 50oC, 2% enzyme addition. Data points are mean values ± standard deviation. n = 2 to 4. 87

Figure 4.10: NPN concentration at different pHs after (A) 60 minutes and (B) 120 minutes of hydrolysis time. Mean value ± standard deviation for n=4 trials. 89
Figure 4.11: NPN content (g/100 g dry sample) of hydrolysate at different processing temperatures and 2% enzyme concentration. Data points are mean values ± standard deviation, n = 2 to 6. 91
Figure 4.12: NPN concentration (g/100 g dry sample) in hydrolysates after 120 minutes at different reaction temperatures, with 2% enzyme addition. Data points are mean values ± standard deviation, n = 2 to 6.. 92
Figure 4.13: SDS –PAGE patterns of beef muscle proteins after hydrolysis with 2% w/w ZyactinaseTM at different temperatures for different reaction times. Lanes 1, 2 and 3: T = 40°C, 50 °C and 60 °C , respectively, 0 minutes, without enzyme; lane 4 : molecular weight marker; Lane 5: T= 40°C , t= 5 minutes; Lane 6: T = 50°C , t = 5 minutes; Lane 7: T = 60°C , t = 5 minutes; Lane 8: T = 40°C , t = 90 minutes; Lane 9: T = 50°C , t = 90 minutes; Lane 10: T = 60°C , t = 90 minutes. (T = temperature, t = time) 93
Figure 4.14: SDS –PAGE of beef muscle after reaction with no enzyme at 60 °C for different times (0, 30, 60 and 90 minutes). Lane 1,2,3,4, 6,7 and 8 : t= 1 minute, 30 minutes, 60 minutes, 90 minutes, 120 minutes , 180 minutes and 0 minute, respectively. Lane 5: molecular weight marker... 94
Figure 4.15: SDS -PAGE of beef muscle hydrolysate with 2% enzyme concentration for different hydrolysis times and temperatures: A: 60°C, B: 50°C, C: 40°C. 95
Figure 4.16: NPN content (g/100 g dry sample) at different enzyme concentrations and different processing temperatures. A: 40 °C, B: 50 °C, C: 60 °C. Data points are mean values ± standard deviation, n = 4 to 6.. 97
Figure 4.17: Changes in pH during hydrolysis at difference enzyme concentrations: (A) no enzyme addition, (B) 1% enzyme concentration, (C) 2% enzyme concentration, (D) 4% enzyme concentration. Data points are mean values ± standard deviation, n = 2 . 98
Figure 4.18: SDS-PAGE: Beef muscle after hydrolysis with ZyactinaseTM enzyme at different enzyme concentrations for 0 & 120 minutes (1 mg/ml protein per load). Lane 1: 60°C, 0 minute, without enzyme; Lane 2: 60°C,120 minutes, without enzyme; Lane 3: 60°C, 1 minute, 1%w/w enzyme; Lane 4: 60°C,120 minutes, 1%w/w enzyme; Lane 5: Molecular weight marker; Lane 6: 60°C,1 minute, 4%w/w enzyme; Lane 7: 60°C,120 minutes, 4%w/w enzyme; Lane 8: 60°C, 1 minute, 2%w/w enzyme; Lane 9: 60°C, 120 minutes, 2%w/w enzyme. 99

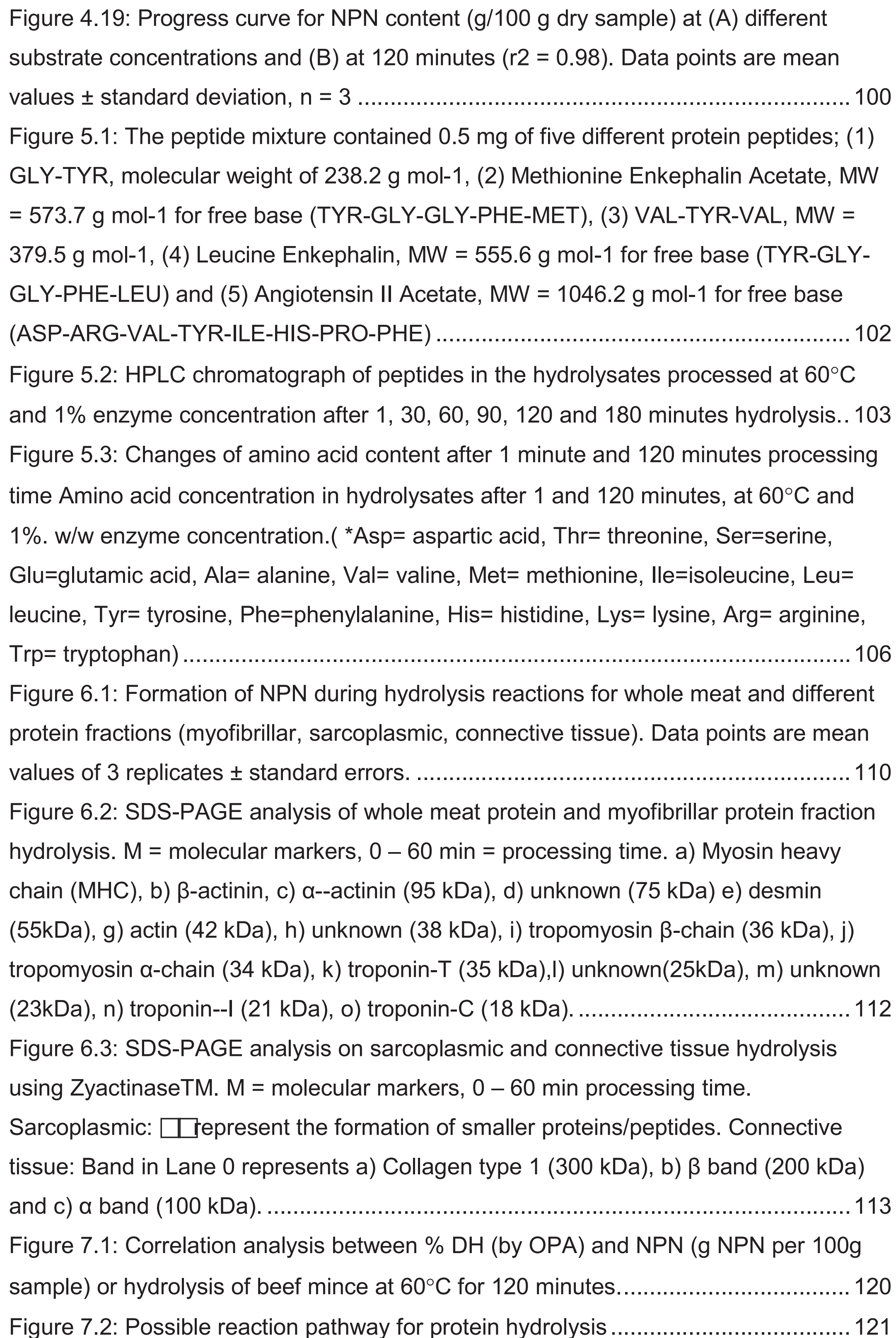

Figure 4.19: Progress curve for NPN content (g/100 g dry sample) at (A) different substrate concentrations and (B) at 120 minutes (r2 = 0.98). Data points are mean values ± standard deviation, n = 3 100

Figure 5.1: The peptide mixture contained 0.5 mg of five different protein peptides; (1) GLY-TYR, molecular weight of 238.2 g mol-1, (2) Methionine Enkephalin Acetate, MW = 573.7 g mol-1 for free base (TYR-GLY-GLY-PHE-MET), (3) VAL-TYR-VAL, MW = 379.5 g mol-1, (4) Leucine Enkephalin, MW = 555.6 g mol-1 for free base (TYR-GLY-GLY-PHE-LEU) and (5) Angiotensin II Acetate, MW = 1046.2 g mol-1 for free base (ASP-ARG-VAL-TYR-ILE-HIS-PRO-PHE) 102

Figure 5.2: HPLC chromatograph of peptides in the hydrolysates processed at 60°C and 1% enzyme concentration after 1, 30, 60, 90, 120 and 180 minutes hydrolysis.. 103

Figure 5.3: Changes of amino acid content after 1 minute and 120 minutes processing time Amino acid concentration in hydrolysates after 1 and 120 minutes, at 60°C and 1%. w/w enzyme concentration.(*Asp= aspartic acid, Thr= threonine, Ser=serine, Glu=glutamic acid, Ala= alanine, Val= valine, Met= methionine, Ile=isoleucine, Leu= leucine, Tyr= tyrosine, Phe=phenylalanine, His= histidine, Lys= lysine, Arg= arginine, Trp= tryptophan) 106

Figure 6.1: Formation of NPN during hydrolysis reactions for whole meat and different protein fractions (myofibrillar, sarcoplasmic, connective tissue). Data points are mean values of 3 replicates ± standard errors. 110

Figure 6.2: SDS-PAGE analysis of whole meat protein and myofibrillar protein fraction hydrolysis. M = molecular markers, 0 – 60 min = processing time. a) Myosin heavy chain (MHC), b) β-actinin, c) α--actinin (95 kDa), d) unknown (75 kDa) e) desmin (55kDa), g) actin (42 kDa), h) unknown (38 kDa), i) tropomyosin β-chain (36 kDa), j) tropomyosin α-chain (34 kDa), k) troponin-T (35 kDa),l) unknown(25kDa), m) unknown (23kDa), n) troponin--I (21 kDa), o) troponin-C (18 kDa). 112

Figure 6.3: SDS-PAGE analysis on sarcoplasmic and connective tissue hydrolysis using ZyactinaseTM. M = molecular markers, 0 – 60 min processing time. Sarcoplasmic: □□represent the formation of smaller proteins/peptides. Connective tissue: Band in Lane 0 represents a) Collagen type 1 (300 kDa), b) β band (200 kDa) and c) α band (100 kDa). 113

Figure 7.1: Correlation analysis between % DH (by OPA) and NPN (g NPN per 100g sample) or hydrolysis of beef mince at 60°C for 120 minutes. 120

Figure 7.2: Possible reaction pathway for protein hydrolysis 121

Figure 7.3: Progress curves for hydrolysis at 60°C at different enzyme concentrations (%w/w) monitored by A: OPA method (%DH) and B: SN-TCA method (NPN). Data points are mean values ± standard deviation, n = 2 - 4. 122
Figure 7.4: Effect of addition of fresh enzyme during hydrolysis. Data points are mean values ± standard deviation, n=4. Hydrolysis at 60°C and 2% w/w enzyme concentration. 124
Figure 7.5: Hydrolysis curve of meat hydrolysis using 2% w/w Zyactinase at 60°C and 360 minutes. 125
Figure 7.6: Effect of addition of fresh enzyme during hydrolysis. Data points are mean values ± standard deviation, n=4 at Figure 7.4 : Hydrolysis curve at 60°C ,2% w/w and up to 300 minutes hydrolysis time. 126
Figure 7.7: Progress curve for amino acid released during hydrolysis reactions at 60°C and substrate concentration 22.2% protein for 180 minutes. Control – no enzyme addition and 2%w/w enzyme added. Data points are mean values ± standard deviation, n=2 to 4. 128
Figure 7.8: Determination of initial velocity (V_0) for the linear part of the hydrolysis progress curve at different substrate concentrations and 2% w/w enzyme concentration. Substrate concentration % w/w protein: A= 2.0%, B= 4.0%, C=6.0%, D=8.0%, E=10.0%, F= 15.0% and G = 22.2%. Data points are mean values ± standard deviation, n = 4. 129
Figure 7.9: Determination of initial velocity (V_0) for the linear part of the hydrolysis progress curve at different substrate concentrations and 4% w/w enzyme concentration. Substrate concentration % w/w protein: A= 2.0%, B=4.0%, C=6.0%, D=8.0%, F=10.0%, G= 12.0%, H = 15.0%, I=22.2% . Data points are mean values ± standard deviation, n = 4. 130
Figure 7.10: Determination of initial velocity (V_0) for the linear part of the hydrolysis progress curve at different substrate concentration and 7.5% w/w enzyme concentration. Substrate concentration % w/w protein: A= 2.0%, B= 4.0%, C=6.0%, D=8.0%, E=10.0%, F= 15.0% and G = 22.2%.Data points are mean values ± standard deviation, n = 4. 131
Figure 7.11: Initial velocity (Serine NH_2 (meq g^{-1} min^{-1})) from at different substrate concentrations (%S = 2, 4 and 7.5 w/w protein). Data points are mean values ± standard deviation, n= 4.. 134

Figure 7.12: Lineweaver-Burk transformation of the progress curve at different substrate concentrations (1/S where S = %S or [S], 60°C. Enzyme concentrations 2, 4 and 7.5% w/w. Data points are mean values ± standard deviation, n= 4. 135
Figure 7.13: Enzyme rate with predicted hyperbolic kinetic pattern on progress curve at different substrate concentrations (1/S where S =% S or [S], 60°C. Enzyme concentrations 2, 4 and 7.5% w/w. Data points are mean values ± standard deviation, n= 4. 136
Figure 7.14: Initial rate of amino acid released at different enzyme concentrations (%w/w) and 10% (w/w protein) substrate concentration. 137

List of Tables

Table 2.1: Proximate composition of meat from various sources expressed as a percentage of weight of edible portion (Fennema et al., 2008)....................................4
Table 2.2: Amino acid composition in fresh meat 19
Table 3.1: Preparation of resolving and stacking gels for analytical and preparative SDS-PAGE. (Bio-Rad formulation, Bio-Rad Laboratories Inc., USA) 63
Table 3.2: Nitrogen content of the starting material for meat protein fraction hydrolysis 65
Table 3.3: The chromatographic gradient programme used for peptides separation ...66
Table 3.4: The chromatographic gradient programme used for amino acid separation68
Table 4.1: Proximate composition of minced beef used for all experiments. Results are mean values of 10 replicates ± standard deviation...74
Table 4.2: Proximate composition of meat hydrolysate Results are mean values 3 replicates ± standard deviation. 76
Table 4.3: Moisture content (g water per 100 g sample) during hydrolysis at 40°C (Results are mean ± standard deviation, n = 3)...77
Table 6.1: Mass of fractions recovered after separation of beef meat into different fractions. 108
Table 6.2: Composition of diluted hydrolysis solutions for each meat fraction. Results are mean values of 3 replicates ± standard deviation..109
Table 7.1: Michaelis-Menten K_m and V_{max}values for meat hydrolysis at different Zyactinase^{TM} enzyme concentrations – from Lineweaver-Burk transformations. 133

List of abbreviations

The following table describes the significance of various abbreviations and acronyms used throughout the thesis.

Abbreviation	Meaning
-COOH	Carboxyl group
-NH2	Amino group

ADP	Adenosine diphosphate
APS	Ammonium persulfate
ARI	Allegenicity reduction index
ATP	Adenosine triphosphate
C	Weight percentage of cross linker
Ca^{2+}	Calcium
Da	Dalton
DH	Degree of hydrolysis
DM	Dry matter
DTNB	Ellman's Reagent (5,5'-dithio-bis-[2-nitrobenzoic acid])
DTT	Dithiothreitol
EC	Emulsification capacity
F-actin	Filament actin
G- actin	Globular actin
HMM	Heavy meromyosin
HPLC	High performance liquid chromatography
IgE	Immunoglobulin E
k_{cat}	Turnover number
k_m	Michaelis constant
LMM	Light meromyosin
N	Nitrogen
NPN	Non-protein nitrogen

OPA	o-phthalaldehyde
pI	Isoelectric point
SDS	Sodium dodecylsulfate
SDS - PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SH	Sulfhydryl group
T	Total monomer concentration
t	Time
TCA	Trichloroacetic acid
TFA	Trifluoroacetic acid
TN-C	Troponin C
TN-I	Troponin I
TN-T	Troponin T
T_S	Shrink temperature
v/v	Volume over volume
V_0	Initial velocity
V_{max}	Maximum velocity
w/v	Weight per volume