

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.



Actinidin- the predominant protease in kiwifruit

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

Master of Philosophy

in

Food Technology

at Massey University, Manawatū,

New Zealand

Dongfang Chao

2016



Studies serve for delight, for ornament, and for ability.

----- Francis Bacon

ABSTRACT

Kiwifruit protein (actinidin) has been widely known as a protease. Kiwifruit protein has the potential of utilization in food industry as an enzyme that aids food digestion.

In this project, the soluble kiwifruit proteins were extracted from fresh Hayward and SunGold kiwifruit. Soluble kiwifruit proteins were analysed by the Hartree-Lowry method, SDS-PAGE, enzyme activity determination, ion-exchange chromatography and mass spectrometry. Anti-actinidin antibodies were raised by the injection of purified actinidin into rabbits. The main soluble kiwifruit protein was recognized by anti-actinidin antibodies using Western blot. Moreover, the effects of post-harvest storage on protein content, total enzyme activity and specific enzyme activity were investigated. Comparable studies on both Hayward and SunGold kiwifruit were also carried out in this project.

The results showed that Hayward and SunGold kiwifruit had a similar protein content. However, the total enzyme activity of Hayward kiwifruit was about 8 times higher than that of SunGold kiwifruit. The protein with enzyme activity (active actinidin) had a molecular weight of about 27 kDa according to SDS-PAGE and was one of main soluble proteins in Hayward and SunGold kiwifruit. This protease was purified from fresh kiwifruit by anion-exchange chromatography. A polyclonal antibody against actinidin was successfully generated in a rabbit using purified actinidin. Protein with a molecular weight of 27 kDa was recognized by the anti-actinidin antibodies. Post-harvest storage at 1 °C for up to 12 weeks significantly increased the total and specific enzyme activities of SunGold kiwifruit ($P < 0.05$). By contrast, the total and specific enzyme activities of Hayward kiwifruit had a significant decrease after 16 weeks'

Abstract

storage ($P < 0.05$). Hayward kiwifruit had no significant changes in protein content after storage ($P < 0.05$) while the protein content of SunGold kiwifruit fluctuated in a range from 5.04 to 5.84 mg/mL during post-harvest storage.

This study may help to understand the nature of kiwifruit proteins with enzyme activity, which contributes to a full understanding of the health benefits of kiwifruit.

ACKNOWLEDGEMENTS

I would like to express my special gratitude to my chief supervisor Dr. Mike Boland for his teaching, guidance, support, and patience. It is impossible for me to learn so much without Dr. Boland's kindness and encouragement. I also appreciate Dr. Juliet Ansell from Zespri Group Limited, Dr. Simon Loveday from Riddet Institute and Dr. Gill Norris from Institute of Fundamental Sciences, who have also provided many valuable suggestions along the way.

All the staff and students at Riddet Institute are very much appreciated. The inspirations from the nice and diversity environment are very important to my journey of study. I also thank all professors, staff and students at Massey University who have helped me during my study.

Special thanks go to my family.



TABLE OF CONTENTS

ABSTRACT	i
ACKNOWLEDGEMENTS.....	iii
TABLE OF CONTENTS.....	v
LIST OF FIGURES	ix
LIST OF TABLES	xi
ABBREVIATIONS	xiii
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	3
2.1 Kiwifruit.....	3
2.2 Major soluble proteins in kiwifruit.....	3
2.2.1 Purification of Actinidin from Kiwifruit.....	4
2.3 The Biochemical Analysis of Actinidin.....	5
2.3.1 Molecular Analysis of Actinidin	5
2.3.2 Actinidin amino acid sequences and molecular structures.....	9
2.3.3 Actinidin Identification in Kiwifruit	10
2.3.4 Actinidin Characterizations by Mass Spectrometry.....	13
2.3.5 Actinidin activity.....	15
2.4 Actinidin and kiwifruit post-harvest storage condition.....	16
2.5 Application of Actinidin in Food Industry.....	17
2.6 Actinidin and Health Benefits	17
CHAPTER 3 MATERIALS AND METHODS	19
3.1 Materials.....	19

Table of Contents

3.2 Chemicals	19
3.3 Kiwifruit	19
3.4 Methods	19
3.5 Actinidin Extraction Methods.....	19
3.6 Protein Concentration Determination.....	20
3.7 Enzyme activity determination	26
3.8 Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS-PAGE)	27
3.9 Stability of Actinidin in Kiwifruit Extracts at different pHs	27
3.10 Actinidin Purification by Ion-exchange Chromatography.....	27
3.11 Mass Spectrometry Analysis	28
3.12 Raising Polyclonal Antibody against Actinidin.....	29
3.12.1 Immunization	29
3.12.2 Serum Preparation	29
3.12.3 Immunoblotting	29
3.13 Actinidin and Kiwifruit Post-Harvest Storage Conditions	30
3.13.1 SunGold Kiwifruit.....	30
3.13.2 Green Kiwifruit	30
3.14 Statistical analysis	31
CHAPTER 4 ACTINIDIN EXTRACTION AND ENZYME ACTIVITY OF KIWIFRUIT	33
4.1 Introduction	34
4.2 Results and discussion.....	35
4.2.1 Protein content of kiwifruit extract	35
4.2.2 SDS-PAGE protein patterns of kiwifruit extract.....	37

4.2.3 Enzyme activity of different cultivars of kiwifruit.....	39
4.2.4 Effect of pH and incubation on enzyme activity	40
4.3 Conclusions	41
CHAPTER 5 ACTINIDIN PURIFICATION AND IDENTIFICATION	43
5.1 Introduction	44
5.2 Results and discussion.....	46
5.2.1 Ion-exchange chromatography of kiwifruit proteins.....	46
5.2.2 SDS-PAGE of Hayward kiwifruit soluble proteins	48
5.2.3 Actinidin Analysis by Mass Spectrometry (Hayward kiwifruit).....	49
5.2.4 Anti-rabbit actinidin antibody generation	53
5.3 Conclusions	55
CHAPTER 6 EFFECT OF POST-HARVEST STORAGE ON THE ACTINIDIN ACTIVITY AND PROTEIN CONTENT	57
6.1 Introduction	58
6.2 Results and discussion.....	59
6.2.1 Variability of enzyme activity among fruits.....	59
6.2.2 Effect of post-harvest storage on actinidin enzyme activity of SunGold kiwifruit from different growers.....	59
6.2.3 Effect of post-harvest storage on actinidin enzyme activity of Hayward kiwifruit.....	62
6.2.4 Changes of Soluble Protein Content with Storage Time.....	64
6.3 Conclusions	66
CHAPTER 7 OVERALL CONCLUSION AND DISCUSSION	67
7.1 Overall Conclusions and Discussion	67
7.1.1 Protein Determination of Kiwifruit Extract.....	67

Table of Contents

7.1.2 Enzyme Activity.....	68
7.1.3 Kiwifruit Soluble Protein Composition and Actinidin Purification.....	68
7.1.4 Effect of Post-harvest Storage on Enzyme Activity and Protein Content	69
7.2 Recommendation for future work	70
7.2.1 Separation and identification of actinidin isoforms	70
7.2.2 Effect of ripening and postharvest on enzyme activity of actinidin.....	71
7.3 Implications of the research	71
CHAPTER 8 REFERENCES	73

LIST OF FIGURES

FIGURE 2-1 COMPARISON OF AMINO ACID SEQUENCES OF ACTINIDIN ISOFORMS FROM KIWIFRUIT.....	8
FIGURE 2-2 AMINO ACID SEQUENCE OF ACTINIDIN FROM KIWIFRUIT	9
FIGURE 3-1 STANDARD CURVE FOR HARTREE-LOWRY METHOD	22
FIGURE 3-2 STANDARD CURVES FOR BRADFORD METHOD.	23
FIGURE 3-3 STANDARD CURVES FOR BCA METHOD.....	25
FIGURE 4-1 ANALYSIS OF SOLUBLE PROTEIN CONTENT IN FRESH KIWIFRUIT BY SDS-PAGE UNDER REDUCING CONDITIONS.....	38
FIGURE 4-2 ENZYME ACTIVITY OF EXTRACTS OF GREEN AND GOLD KIWIFRUIT.....	39
FIGURE 4-3 ANALYSIS OF ACTINIDIN ACTIVITY OVER TIME IN DIFFERENT PH ENVIRONMENTS.....	41
FIGURE 5-1 ION-EXCHANGE CHROMATOGRAPHY OF HAYWARD KIWIFRUIT SOLUBLE PROTEINS ON DEAE-SEPHADEX A-25.....	47
FIGURE 5-2 SDS-PAGE ANALYSIS OF ACTINIDIN FRACTIONS PURIFIED BY ION-EXCHANGE CHROMATOGRAPHY	48
FIGURE 5-3 MASS SPECTRUM OF FRACTION (#46) WITH SPECIFIC ENZYME ACTIVITY OF 9.6	50
FIGURE 5-4 MASS SPECTRUM OF FRACTION (#41) WITH SPECIFIC ENZYME ACTIVITY OF 8.1	51
FIGURE 5-5 MASS SPECTRUM OF FRACTION (#37) WITH SPECIFIC ENZYME ACTIVITY OF 6.6	52
FIGURE 5-6 WESTERN BLOT ANALYSIS OF PROTEIN EXTRACTED FROM HAYWARD KIWIFRUIT AND SUNGOLD KIWIFRUIT	54

List of Figures

FIGURE 6-1 SPECIFIC ENZYME ACTIVITY OF VARIABILITY OF DIFFERENT INDIVIDUAL KIWIFRUIT	59
FIGURE 6-2 SPECIFIC ENZYME ACTIVITY OF SOLUBLE PROTEIN FROM HAYWARD KIWIFRUIT (A) AND ENZYME ACTIVITY OF HAYWARD KIWIFRUIT (B) AT COMMERCIAL HARVEST TIME (DAY 0) AND AFTER 1, 2, 4, 8, 12 AND 16 WEEKS' STORAGE.	63
FIGURE 6-3 PROTEIN CHANGES IN HAYWARD KIWIFRUIT EXTRACTION DURING POST-HARVEST STORAGE AT 1°C.....	65

LIST OF TABLES

TABLE 4-1 PROTEIN CONTENT MEASURED BY DIFFERENT METHODS.....	36
TABLE 6-1 SPECIFIC ENZYME ACTIVITY OF SUNGOLD KIWIFRUIT	60
TABLE 6-2 ENZYME ACTIVITY OF SUNGOLD KIWIFRUIT.....	61
TABLE 6-3 PROTEIN CHANGES IN SUNGOLD KIWIFRUIT EXTRACTION DURING POST-HARVEST STORAGE.....	65



ABBREVIATIONS

N- α -CBZ-lysine- pNP	N- α -carbobenzoxy-L-lysine-p-nitrophenyl ester
DEAE	Diethylaminoethyl
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
cDNA	Complementary DNA
mRNA	Messenger RNA
BSA	Bovine serum albumin
%	All percentages are weight/volume (w/v) unless otherwise stated
Tris	Tris (hydroxymethyl) amino methane

Amino acid abbreviations:

Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic Acid
Cys	Cysteine
Gln	Glutamine
Glu	Glutamic Acid
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine

Abbreviations

Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine