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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

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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.



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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