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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

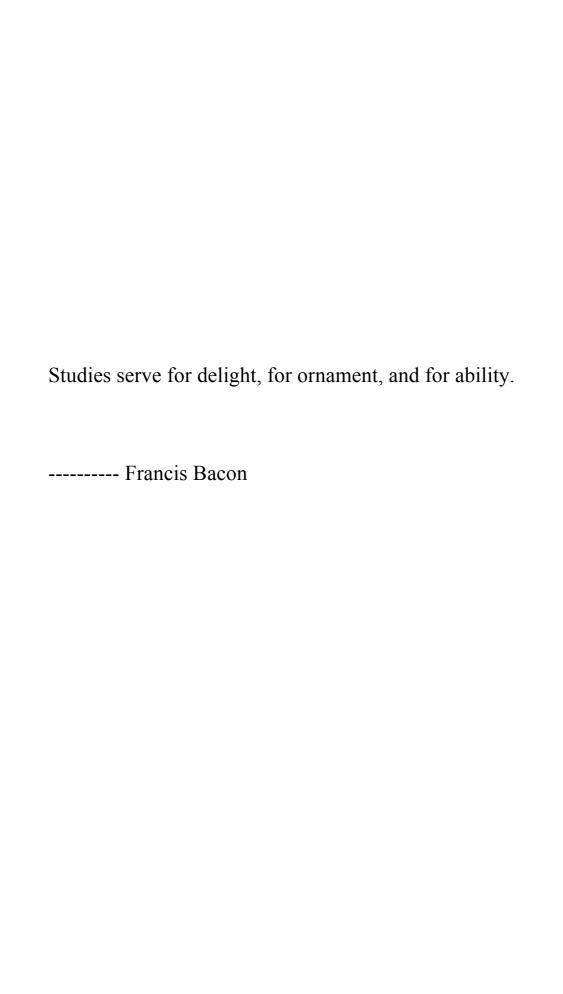
at Massey University, Manawatū,

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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'

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.

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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 (hydroxymethyl) amino methane

Amino acid abbreviations:

Ala Alanine

Arg Arginine

Asn Asparagine

Asp Aspartic Acid

Cys Cysteine

Gln Glutamine

Glu Glutamic Acid

Glycine

His Histidine

Ile Isoleucine

Leu Leucine

Lys Lysine

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Abbreviations

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