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BEEF HYDROLYSIS BY ZYACTINASE™
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.
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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.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
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<tbody>
<tr>
<td>-COOH</td>
<td>Carboxyl group</td>
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<tr>
<td>-NH2</td>
<td>Amino group</td>
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xviii
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium persulfate</td>
</tr>
<tr>
<td>ARI</td>
<td>Allegenicity reduction index</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>C</td>
<td>Weight percentage of cross linker</td>
</tr>
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<td>Calcium</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
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<td>DH</td>
<td>Degree of hydrolysis</td>
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<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>DTNB</td>
<td>Ellman’s Reagent (5,5'-dithio-bis-[2-nitrobenzoic acid])</td>
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<td>Dithiothreitol</td>
</tr>
<tr>
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<td>Emulsification capacity</td>
</tr>
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<td>Filament actin</td>
</tr>
<tr>
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<td>Globular actin</td>
</tr>
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<tr>
<td>OPA</td>
<td>o-phthalaldehyde</td>
</tr>
<tr>
<td>pI</td>
<td>Isoelectric point</td>
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<td>SDS</td>
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<td>Sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
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<tr>
<td>T_s</td>
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<tr>
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<td>Volume over volume</td>
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<td>V_0</td>
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<tr>
<td>V_max</td>
<td>Maximum velocity</td>
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<tr>
<td>w/v</td>
<td>Weight per volume</td>
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