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APPLICATION FOR APPROVAL TO EMBARGO A THESIS

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Programme of study:

Thesis title: Beef hydrolysis using Zyactinase enzyme

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

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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

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THE ENGINE OF THE NEW NEW ZEALAND

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^{TM}, 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^{TM} 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^{TM} 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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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