

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.

**Effect of Mechanical Work on the Meat Used for Making
Reformed Meat Products**

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

Doctor of Philosophy

in

Food Technology

at Massey University, Palmerston North,

New Zealand

ISMAIL FITRY MOHAMMAD RASHEDI

2014

Abstract

Tumbling, a process commonly used during production of reformed meat, applies mechanical work against the meat pieces to break down the meat structure, enhance brine absorption and extract solubilized myosin to the meat surface. The myosin acts as glue to bind meat pieces together when heated. The work done in the tumbler is currently unquantifiable and its relationships with total protein and myosin extraction and binding strength (Tensile Adhesive Strength, TAS) of two meat pieces are unknown.

Much of this project was allocated to developing and evaluating an instrument called the Impact and Friction Mechanical Robot (IFMR), which is able to repeatedly apply a desired impact and to vary the rate of repeated impacts and the time gap between each impact. The degree of sample compression could also be varied. The work done as a consequence of the hitting process can be calculated for each individual hit and summed to give the total work impacted on the meat.

Four groups of 20 mm³ meat cubes were prepared for the hitting treatments. One group was used as the control while the other three were pre-soaked in 0.396, 0.713 or 1.146 mol/L of brine consisting of NaCl and salts of phosphate. The meat cubes were hit so maximum impact force was 10 N with an average 0.665 s between each hit for 0, 400, 800, 1200, 1600 or 2000 hits. The exudate on the hit meat surface was scraped off and examined for total protein and myosin.

The total protein extracted was not influenced by the work ($p=0.764$) applied on meat cubes pre-soaked in different concentrations of brine ($p=0.123$). Myosin extracted increased with total work done ($p=0.006$) on the meat and concentration of brine ($p<0.001$) used for soaking.

Two meat cubes were attached together at the hit surfaces, cooked at 70 °C with a 250 g weight applied, and tested for tensile adhesive strength (TAS). The TAS between meat pieces increased with increased total work ($p=0.0001$) done on the meat and increased brine concentration ($p<0.001$) for pre-soaking. The TAS also increased as myosin concentration increased ($p=0.001$). A good TAS of the meat pieces could be achieved by adequately solubilising the myosin using brine and applying sufficient total work to the meat pieces.

Acknowledgements

Glory and Praise be to Allah, the most gracious and merciful.

This journey was long, so long that I thought it will never end. If not for the help and guidance of my supervisory committee and the faculty members, and spiritual and emotional support of my beloved family and friends, I would not be able to finish it.

I would like to express my deepest gratitude to my supervisors; Prof Tony Paterson, Assoc. Prof Brian Wilkinson and Assoc. Prof Roger Purchas. Tony has been a great supervisor. He always listens to the problems I had in whatever aspect, either in the study or personal life. He has been like a teacher, a father and a friend at the same time. Brian is an easily approach supervisor. His knowledge is abundant and he's willing to share his idea anytime I needed. Roger has been a very patient supervisor. He has guided me to the very detail of my work making sure that I produced a well presented thesis. They were always by my side passionately helping me to go through my study. I could not thank them enough for their guidance.

A special thanks to Dr Rory Flemmer for his supervision in building the IFMR. A big thank you to all the staff in SEAT and IFNHH; Stan, Clive, Kerry and Ian from the workshop, Nick from the IT department, Bruce and Anthony from the electronics department, Ann-Marie, Judy and Julia from micro suite lab, Steve and Michelle from food chemistry lab, Gary from the pilot plant, Warwick from the product development lab and John Edwards who I can find him everywhere in the faculty.

Thank you to my officemates, Shailesh, Konrad, Tawan, Rhonda and Adeliné for their friendship and company. Thank you to all Malaysian friends in Palmy for all the great moments we shared together. I could not spell their names here because there were too many of them but I am sure they know who they are.

Thank you to Ministry of Higher Education of Malaysia for the financial sponsorship. Thank you to all the lecturers and staff in FSTM, UPM who have been supporting me.

Thank you to my Emak and Abah, Mami and Ayah, and all the siblings for their patience when I was away from home. Last but not least, thank you to my beloved wife Nor Khaizura and my sons Uzair Aqil and Uwais Akif for the love, patience and support for me throughout this journey.

Table of Contents

Abstract.....	I
Acknowledgements	II
Table of Contents.....	III
List of Tables	VII
List of Figures.....	VIII
List of Abbreviations.....	XII
Nomenclature	XIII
CHAPTER 1.....	1
CHAPTER 2.....	5
2.1. Reformed and restructured meat products.....	6
2.2. Tumbling and Massaging.....	7
2.3. The mechanics of falling meat in the tumbler.....	8
2.4. The effects of time, speed, and pattern of tumbling on product characteristics	9
2.4.1. Time of processing.....	9
2.4.2. Speed of processing.....	10
2.4.3. Continuous or intermittent tumbling	11
2.5. Effect of physical treatments on meat structure and brine absorption	12
2.6. Proteins for Binding Meat Pieces	16
2.7. Factors Affecting Muscle Protein Solubility and Extractability	19
2.7.1. Salt and Type of Phosphate.....	19
2.7.2. Animal species and muscle type	23
2.8. The effects of fibre orientation and heating treatments on binding strength	25
2.8.1. Meat fibre orientation	25
2.8.2. The effects of heat treatments on gel-network formation by myosin.....	26
2.9. Effects of mechanical work applied to meat and binding strength.....	27
2.10. Conclusions and recommendations	28

CHAPTER 3.....	31
3.1. Introduction	32
3.2. Design and Construction.....	32
3.2.1. Framework and compartments	32
3.2.2. Computers and electronics	36
3.3. Code and functions.....	37
3.3.1. Controlling the stepper motor movement.....	41
3.4. Measuring the force and time.....	43
3.5. Calculating the distance	44
3.6. Calculating the work.....	47
3.6.1. Matching time using Look-up feature in Excel.....	48
3.6.2. Fitting the force with the distance	50
3.7. Distance measurement by a potentiometer variable resistor.....	54
3.8. Replacing the load cell with a hydraulic pressure sensor.....	59
3.9. Tests of IFMR performance using meat samples.....	62
3.10. Conclusions.....	67
CHAPTER 4.....	69
4.1. Introduction.....	70
4.2. Preparation of meat cubes	70
4.3. Meat soaking.....	70
4.4. Meat hitting	71
4.5. Collecting and analysing protein from meat	71
4.5.1. Washing methods.....	71
4.5.2. Scraping methods.....	73
4.5.3. Bradford protein assay	73
4.5.4. Electrophoresis (SDS-PAGE) analysis.....	74
4.5.5. Identifying the suitable method for collecting exudate	79
4.6. Results and discussion.....	79

4.7. Conclusions.....	83
CHAPTER 5.....	85
5.1. Introduction.....	86
5.2. Meat cooking and adhesion testing.....	86
5.2.1. Wrapping the meat cubes.....	86
5.2.2. Mould.....	87
5.2.3. Cooking procedure.....	88
5.2.4. Tensile adhesive strength (TAS) analysis.....	88
5.2.5. Experimental design and statistical analysis.....	89
5.3. Results and discussion.....	90
5.4. Conclusions.....	91
CHAPTER 6.....	93
6.1. Introduction.....	94
6.2. Materials and methods.....	94
6.2.1. Meat soaking.....	94
6.2.2. Meat hitting.....	96
6.2.3. Weight gain after soaking.....	96
6.2.4. Hitting loss measurement.....	96
6.2.5. Cooking loss measurement.....	96
6.2.6. Firmness.....	97
6.2.7. Measuring TAS of intact meat.....	97
6.2.8. Total protein and myosin analysis of hit-treated meat cubes and tensile adhesive strength of the junction of attached meat cubes.....	97
6.2.9. Design and statistical analysis.....	98
6.3. Results and Discussion.....	99
6.3.1. TAS of intact meat.....	99
6.3.2. Effects of brine strength and total work on weight gain, hitting loss, cooking loss and firmness of 20 mm cubes of beef semitendinosus muscle.....	100

6.3.3. Effects of brine concentration and applied work on the exudate and binding strength of a pair of cooked 20 mm cubes of beef semitendinosus muscle	106
6.4. Conclusions	117
CHAPTER 7.....	119
7.1. Conclusions	120
7.2. Future recommendations.....	121
References.....	123
Appendices.....	133

List of Tables

Table 2.1. The distribution of muscle fibre types for different muscles and different species.	24
Table 4.1. List of chemicals for preparing the gel.	75
Table 4.2. Formula for one layer of casting gel.	76
Table 4.3. Formula for one layer of stacking gel.	77
Table 4.4. Sample preparation formula.	78
Table 6.1. Formulations used for making the brine consisting of salt (NaCl) and phosphates and the brine ionic strength.	95
Table 6.2. Design of the hitting treatment applied to the meat cubes.	98
Table 6.3. The effect of ionic strength of the brine on weight gain of meat cubes.	100
Table 6.4. The linear or quadratic equation produced with R^2 and Relative Standard Deviation (RSD). The factors were fitted similar to the sequence in the equation. The significance levels of the items involved are given at the relevant places in the text.	105
Table 6.7. The linear equations produced with R^2 and Relative Standard Deviation (RSD). The factors were fitted in the sequence shown in the equations. The significance levels of the items involved are given at the relevant places in the text.	110
Table 6.5. Matched pairs of values for TAS and total protein that have been paired because they were associated with similar or near applied averaged total work (J) \pm SD values (4 values for TAS and 2 values for total protein).	111
Table 6.6. Matched pairs of values for TAS and exudate myosin concentration that have been paired because they were associated with similar or near applied averaged total work (J) \pm SD values (4 values for TAS).	114
Table 6.8. The linear equations produced with R^2 and Relative Standard Deviation (RSD). The factors were fitted in the sequence shown in the equations. The significance levels of the items involved are given at the relevant places in the text.	116

List of Figures

Figure 2.1. (a) Structure of the skeletal muscle, (b) The muscle fibre (myofibre) and its contents, (c) myofibrils and myofilaments of the skeletal muscle, (d) One sarcomere of myofibril.....	14
Figure 2.2. (a) Myosin, (b) Actin, Troponin and Tropomyosin.	17
Figure 2.3. Dissociation of actomyosin into actin and myosin (process 1) and depolymerisation of thick filament into myosin monomers (process 2).....	21
Figure 2.4. Two possible orientations of meat fibres during meat binding.....	25
Figure 3.1. The framework and compartments of the IFMR equipment. A, vertical stepper motor, limit switch and movable screw; B, steel plate and aluminium container; C, Hitting Head (HH) used for hitting the meat sample and bending beam load cell; and D, horizontal stepper motor and S-beam load cell.	35
Figure 3.2. Connections and wiring set up between the computers and the equipment. Dashed arrows are commands from the master to the slave computer and then to the stepper motor drivers through the CIO-DIO48 board. Solid arrows are the forces recorded by the load cell, converted from analog to digital via the PCI-DAS6025 board and transferred to the master computer.....	37
Figure 3.3. Flowchart for commands to operate the IFMR for the fixed force method. The increase or decrease of the step number (S_N) can be adjusted as required. Details for the fixed distance method are given in Figure 3.4.....	39
Figure 3.4. Flowchart showed commands to operate the IFMR for the fixed distance method.....	40
Figure 3.5. A) accumulated time against step number (100 steps down) and B) accumulated time against accumulated distance during the process (Section 3.5 for calculation of distance per step).	42
Figure 3.6. A) accumulated time against step number (200 steps down) and B) accumulated time against accumulated distance during the process.	42
Figure 3.7. Calibration curve of reading from the load cell in Binary-Coded Decimal (BCD) and resultant Force (N).....	44
Figure 3.8. Stepper Motor rotation movement and its relation to distance travelled by the vertical arm and Hitting Head (HH).....	45
Figure 3.9. Accumulated distance travelled by HH with number of steps taken by the stepper motor. The steps number represents 400 steps down and 400 steps return. The fitted line (dotted line) was overlaid with $R^2=1$	46

Figure 3.10. The force (N) recorded and distance travelled (mm) against time (ms) using Look-up feature on the spring. The spring was hit for 200 steps down and 200 steps return with average time of 0.65 ms per step.....	49
Figure 3.11. The calculated instantaneous work (J) done on the spring for a single hit. The spring was hit for 200 steps down and 200 steps return for an average of 0.65 ms per step. Total work was 0.0015 J.....	49
Figure 3.12. Modelling distance travelled using Weibull, Normal or Polynomial distributions. Data was collected from hitting the spring for 200 steps down and 200 steps return with an average step time of 0.65 ms.....	51
Figure 3.13. Comparison of curves between actual data and fitted data for distance travelled against time using the General model Gaussian (Mat Lab). The actual data was collected from hitting the spring for 200 steps down and 200 steps return with average time of 0.65 ms per step.	52
Figure 3.14. Force (N) and distance (mm) travelled when spring was hit for 200 steps down and 200 steps up with an average time of 0.65 ms per step.....	53
Figure 3.15. The work (J) done on the spring against time (ms) calculated based on an equation produced from modelling the distance fitted line. The spring was hit for 200 steps down and 200 steps return with average time of 0.65 ms per step. Total work was 0.0108 J.....	53
Figure 3.16. Detail of attaching potentiometer to the back of the stepper motor.....	54
Figure 3.17. A flowchart showing commands in the VB program to collect the data from both load cell and potentiometer and converting them to force, distance and work.	55
Figure 3.18. The calibration curve of the potentiometer output against step number.....	56
Figure 3.19. The force and distance against time for one hit on the spring sample with 1 delay unit.....	57
Figure 3.20. Modelling force and distance for one hit on the spring with delay of 10 units.	57
Figure 3.21. Modelling force and distance for one hit on the spring with delay of 100 units.	58
Figure 3.22. Peak force (N) against the delay unit number during hits on the spring.	59
Figure 3.23. The hydraulic pressure sensor attached to the aluminium block containing hydraulic oil.	60
Figure 3.24. Calibration curve for relationship between reading from pressure sensor in BCD and Force (N).....	61
Figure 3.25. The peak force (N) against number of delay units during hits on spring.....	61
Figure 3.26. Average time per step (400 steps) against delay unit value as set up in DOS..	63

Figure 3.27. Average gap time (s) between each hit with the gap unit set in VB command.	63
Figure 3.28. Force and distance travelled from a single hit on a meat sample hit with 10 N target force with average step time of 0.15 ms and average gap time of 0.665 sec	64
Figure 3.29. Work (J) done on meat sample calculated from a single hit as depicted in Figure 3.28. Total work was 0.0066 J.....	65
Figure 3.30. Pattern of maximum force recorded for 100 cycles of hitting with fixed force at 10 N on meat sample. The initial step number (S_N) was set up at 100 steps and the increase/decrease of step number was set up at 1 unit.....	66
Figure 3.31. Pattern of maximum force recorded for 200 cycles of hitting the meat with a setting of fixed distance on the meat cube rather than to a fixed maximum force. The initial step number was fixed at 200 steps.	67
Figure 4.1. The mesh containing the meat cube was inserted into the 50 mL beaker containing 15 mL 5.8% salt solution.	72
Figure 4.2. Standard curve for the Bradford protein assay.	80
Figure 4.3. Total protein (mg/mm ²) in the exudate collected from meat surface treated with IFMR (800 hits, fixed force at 10 N, average time of 0.15 ms per step, and average of 0.665 sec between each hit) against time of washing (hours).....	81
Figure 4.4. Typical gel from SDS-PAGE electrophoresis of scraped exudate from meat samples with different numbers of hits. Lane 1 and 10 were standard with the 250 kD molecular weight for the highest band. Lane 2, 3, 4, 5, 6 and 7 were meat samples treated with 0, 400, 800, 1200, 1600 and 2000 hits, respectively. The highest band for each meat samples lane was the myosin with a molecular weight detected from 190 to 220 kDa.....	82
Figure 5.1. The mould made of steel to put meat cubes wrapped in pairs to cook. This mould is immersed in a water bath for cooking.	87
Figure 5.2. The weights for the meat with Part "A" as the base and Part "B" is the additional weight.....	88
Figure 5.3. Diagram showing clamps used in the TA-TX2 to hold a pair of 20 X 20 X 20 mm meat cubes to measure the tensile adhesive strength.	89
Figure 5.4. Tensile Adhesive Strength for combined pairs of meat cubes for different presser weights and cooking temperatures. Eight replicates were carried out for each condition. Error bars are the standard error, (a, b, c and d are symbols for significance of differences between weights within each temperature and x, y and z are symbols for significance of differences between temperatures at each weight (different letters show significant differences at $P < 0.05$).....	90

Figure 6.1. The Tensile Adhesive Strength (TAS) intact meat cuboids (n=3) and joined meat cubes (n=2) at each ionic strength of the brine (mol/L). Samples were not treated with any mechanical work.....	100
Figure 6.2. The hitting loss (%) of meat cubes measured against the total work (J) following soaking in four concentrations of brine. Control (non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.	102
Figure 6.3. The cooking loss (%) of meat cubes measured against the total work (J) applied within the four brine-soaking treatments. Control (Non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.	103
Figure 6.4. The firmness (N/m ²) of meat cubes measured against the total work (J) done within their soaking conditions. Control (non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.	104
Figure 6.5. Total protein (mg/mm ²) in the scraped-off exudate from the meat cube surface, plotted against total work (J) applied to the meat for meat cubes subjected to four brine treatments. Control (non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.....	106
Figure 6.6. Myosin density over protein (AU/mg) in the scraped-off exudate from the meat cube surface, plotted against total work (J) applied to the meat for meat cubes subjected to four brine treatments. Control (non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.....	107
Figure 6.7. Effect of brine concentration and work on TAS between pairs of meat cubes. Control (Non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.....	109
Figure 6.8. Tensile Adhesive Strength (TAS) (kPa) of meat cubes plotted against the total protein (mg/mm ²) measured on the meat cube surface. Means (±SD) from Table 6.5. Control (Non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.....	112
Figure 6.9. Tensile Adhesive Strength (TAS) (kPa) of meat cubes plotted against the myosin density over total protein (AU/mg) collected from the meat cube surface. Means (±SD) from Table 6.6. Control (Non-soaked), Brine SP0.396=0.396 mol/L, Brine SP0.713=0.713 mol/L and Brine SP1.146=1.146 mol/L.	115

List of Abbreviations

IFMR	Impact and Friction Mechanical Robot
TAS	Tensile Adhesive Strength
NaCl	Sodium chloride
STPP	Sodium tripolyphosphate
TSPP	Tetrasodium pyrophosphate
SHMP	Sodium hexametaphosphate
SDS PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
HH	Hitting head
VB	Visual Basic
DOS	Disk Operating System
GUI	Graphical User Image
BCD	Binary-Coded Decimal
AU	Arbitrary Unit

Nomenclature

S_R	Scanning rate
S_T	Total data to be scanned
n	Number of hits
H_G	average gap time between hits
H_T	average time per step
F_T	force target
D_T	distance target
S_N	initial step number
k	hit number
F_P	Peak force
F_T	Target force