DECLARATION

The candidate certifies that the thesis entitled "Development of plant-based meat analogues from Manila tamarind (*Pithecellobium dulce*) seeds" submitted to the *School of Engineering, Tezpur University* in partial fulfilment for the award of the degree of *Doctor of Philosophy* in the *Department of Food Engineering and Technology* is a record of research work carried out by me under the supervision of **Prof. Nandan Sit**.

All assistance received from various sources has been appropriately acknowledged. No part of this thesis has been submitted elsewhere for the award of any degree.

Date: 13-11-2025

Place: Tezpur University

(Awanish Singh)

Awarish Tresh

Registration No. TZ203926 of 2023

Department of Food Engineering and Technology

School of Engineering, Tezpur University

Napaam 784028, India

तेजपुर विश्वविद्यालय/ TEZPUR UNIVERSITY

(संसद के अधिनियम द्वारा स्थापित केंद्रीय विश्वविद्यालय)
(A Central University established by an Act of Parliament)
तेजपुर-784028 :: असम/ TEZPUR-784028 :: ASSAM

(सर्वोत्तम विश्वविद्यालय के लिए कुलाध्यक्ष पुरस्कार,2016 औरभारत के 100श्रेष्ठ उच्च शिक्षण संस्थानों में पंचम स्थान प्राप्त विश्वविद्यालय)

(Awardee of Visitor's Best University Award, 2016 and 5th among India's Top 100 Universities, MHRD-NIRF Ranking, 2016)

 Dr. Nandan Sit
 Mobile: +91-9864362377

 Professor
 Phone: 03712-275704

Department of Food Engineering and Technology Email: nandan@tezu.ernet.in

Certificate of the Supervisor

This is to certify that the thesis entitled "Development of plant-based meat analogues from Manila tamarind (*Pithecellobium dulce*) seeds" submitted to the School of Engineering, Tezpur University in part fulfillment for the award of the degree of Doctor of Philosophy in Food Engineering and Technology is a record of research work carried out by Mr. Awanish Singh under my supervision and guidance.

All help received by him from various sources have been duly acknowledged.

No part of this thesis has been submitted elsewhere for award of any other degree.

Date: 13-11-2025 (**Prof. Nandan Sit**)

Place: Tezpur University

ACKNOWLEDGEMENTS

At the outset, I extend my heartfelt gratitude to my supervisor, Prof. Nandan Sit, Department of Food Engineering and Technology, Tezpur University, Tezpur, Assam, for his invaluable guidance, unwavering support, and expert insights throughout the completion of this thesis. His encouragement and thoughtful recommendations have been instrumental in shaping this work from its inception to completion, and I deeply appreciate his efforts in organizing my research systematically and within the prescribed timeframe.

I sincerely thank Hon. Vice-Chancellor Prof. Shambhu Nath Singh, Tezpur University, Tezpur, Assam, for providing me with the opportunity to undertake this research. My gratitude also extends to Prof. S. C. Deka, Dean of the School of Engineering, and Dr. Biren Das, Controller of Examinations, and Prof. L. S. Badwaik, HoD, FET Department, Tezpur University, for their continuous support during my thesis work.

I am profoundly grateful to my Doctoral Committee members, Prof. Brijesh Srivastava, Prof. Tarun K. Maji, and Prof. Mrinal Kumar Das, from the Department of Food Engineering and Technology, the Department of Chemical Sciences and the Department of Physics, Tezpur University, for their valuable suggestions and encouragement at various stages of my research.

My sincere appreciation extends to the members of the Departmental Research Committee, Prof. Bhabesh Deka and Dr. Rupak Mukhopadhyay, for their guidance and assistance throughout my research. I also acknowledge the faculty members of the Department of Food Engineering and Technology, including Prof. Charu Lata Mahanta (Retd.), Prof. Manuj Kr. Hazarika, Prof. B. Srivastava, Prof. L. S. Badwaik, Prof. Poonam Mishra, Dr. Swami Hulle Nishant Rachayya, Dr. Tabli Ghosh, Dr. Soumya Ranjan Purohit, and Dr. Nickhil C., for their invaluable input and encouragement throughout my Ph.D. journey.

I am grateful to the technical staff, Dr. Dipankar Kalita, Dr. Arup Jyoti Das, Mr. Labadeep Kalita, and Mr. Bhaskar J. Kalita, as well as the non-technical staff, Mr. Krishna Borah and Mr. Anjan Keot, for their assistance during my research and departmental work.

I am indebted to the University Grants Commission (UGC) for providing me with financial assistance through the UGC-NET Junior Research Fellowship, and I appreciate the staff of the Research and Development cell at Tezpur University, for diligently handling scholarship-related tasks and addressing any grievances.

I would like to express my sincere gratitude to my batchmates Parismita, Somya, Dr. Beatrice, Dr. Shagufta, Dr. Hemanta, Dr. Amardeep, Thoithoi and Wungshim Zimik, and my dear friends Indrani, Sophia, Reetom, Vickey and Biswajit for their continuous support and encouragement during my research journey.

I extend my heartfelt appreciation to my labmates, Dr. Avinash Kr. Jha, Dr. Mohit Singla, Dr. Ditimoni Dutta, Bhaskar Jyoti Kalita, Bharati, Nipona, Prashant, Pinky, Vilhouphrenuo Zatsu, and the juniors, for their unwavering support throughout my research.

I am deeply grateful to Ms. Archana, Ms. Anutee, and Mr. Rituraj, Research Scholars in the Department of Molecular Biology and Biotechnology, Tezpur University, for their contributions, and I wish to acknowledge the support of all those, whether directly or indirectly involved, whose names may not have been explicitly mentioned.

Lastly, my deepest gratitude goes to my grandparents, Late Thakur Sant Singh Vishen, and Late Mrs. Satyavati Devi, my beloved parents, Mr. Lal Bahadur Singh and Mrs. Lalita Singh, my uncles Mr. Amar Bahadur Singh and Mr. Kunwar Bahadur Singh, my aunts Yashoda Singh and Anita Singh, sisters Archana Singh, Prity Singh and Ayushi Singh, brothers Mr. Ajeet Pratap Singh, Saurabh Singh, Manish Singh, Adarsh Singh and my entire family for their unconditional love, encouragement, care, and unwavering support throughout my Ph.D. journey. Their patience, understanding, and motivation have been invaluable in completing this research.

Above all, I am profoundly grateful to the Almighty for the blessings that have guided me throughout my Ph.D. journey.

Awanish Singh

LIST OF TABLES

Table No.	Title	Page No
Table 2.1	Meat analogue sources and the technology behind their manufacturing	29
Table 2.2	Summary of the ingredients of meat analogues with their	35
Table 2.3	functionality and sources Types of meat analogue production technology with their	42
14010 2.5	associated advantages	12
Table 3.1	Physicochemical properties of Manila tamarind seed flour	91
Table 3.2	Physicochemical and Functional properties of Manila	92
	tamarind seed protein isolate	
Table 3.3	Amino acid content (g/100 g protein) of Manila tamarind	94
	seed protein isolate (MTSPI) compared with soy-protein	
	isolate (SPI) (for reference)	
Table 3.4	Protein quality parameters of the Manila tamarind seed	96
	protein isolate (MTSPI)	
Table 3.5	Water solubility of Manila tamarind seed flour protein	97
	isolate (native and modified)	
Table 3.6	Water and oil absorption capacity of Manila tamarind seed	99
	flour protein isolates (native and modified)	
Table 3.7	L^* , a^* , b^* color values of Manila tamarind seed flour	101
	protein isolate (native and modified)	
Table 3.8	Bulk density of Manila tamarind seed flour protein isolates	103
	and modified protein isolate	
Table 3.9	Particle density of Manila tamarind seed flour protein	105
	isolate (native and modified)	
Table 3.10	Textural properties of Manila tamarind seed protein isolate	107
	solution (native and modified) using back extrusion	
Table 3.11	Least gelation concentration (LGC) of Manila tamarind	109
	seed flour protein-isolate and modified protein isolate	
Table 3.12	Emulsifying activity and emulsion stability of Manila	111
	tamarind seed flour protein isolate (native and modified)	

Table 4.1	Different mixture components with their minimum and	131
	maximum values	
Table 4.2	List of all the dependent and independent variables with	132
	the response values	
Table 4.3	Physicochemical properties of Manila tamarind seed	141
	protein isolate (MTSPI) and jackfruit flour	
Table 4.4	ANOVA table for the quadratic model	145
Table 4.5	L^* , a^* and b^* values of the different runs of the	149
	experimental design	
Table 4.6	Optimized values of the different constituents along-with	155
	predicted and experimental values of the response	
	variables	
Table 4.7	Physicochemical and functional properties of the freeze	157
	texturized protein and reference sample	
Table 4.8	Colour and textural properties of the texturized protein	163
Table 4.9	Physicochemical and functional properties of the	172
	texturized proteins	
Table 4.10	Textural properties of the texturized proteins along with	174
	reference sample	
Table 5.1	List of all the independent variables with their different	189
	combinations	
Table 5.2	Bromelain enzyme activity at various temperatures	194
Table 5.3	Two-way ANOVA table (with interaction) for Lightness	199
	(L^*) value, Redness/Greenness (a^*) value, and	
	Yellowness/Blueness (b*) value of seed protein	
	hydrolysates	
Table 5.4	Lightness value (L^*) of the seed protein hydrolysates	201
Table 5.5	Redness/greenness value (a*) of the seed protein	201
	hydrolysates	
Table 5.6	Yellowness/blueness value (b*) of the seed protein	203
	hydrolysates	
Table 5.7	Amino acid content (g/100 g protein) of Manila tamarind	210
	seed protein isolate (MTSPI) and seed protein hydrolysate	
	(SPH) hydrolysed by bromelain enzyme (0.1%)	

Table 5.8	List of all the independent variables with their different combinations and response variable	214
Table 5.9	•	216
	ANOVA for quadratic model	220
Table 5.10	Comprehensive sensory evaluation of chicken-like flavor	220
	components developed under varying processing	
T 11 5 11	conditions	221
Table 5.11	Optimized values of the different independent variables	221
T 11 5 10	and response variable	221
Table 5.12	Meaty/chicken-like flavor compounds and amino acids by	231
	molecular weight	
Table 6.1	Experimental design based on a two-factor factorial design	246
	with 3 levels of frying temperature and 6 levels of frying	
	time	
Table 6.2	Proximate composition analysis of texturized protein and	253
	reference material	
Table 6.3	Two-way ANOVA (with interaction) for Moisture Content	257
	(% w.b.) and Fat Content (% d.b.) of fried samples	
Table 6.4	Moisture content (moisture content) values (% w.b.) for	258
	fried samples obtained after fat-frying at different	
	temperatures	
Table 6.5	Fat content values (% d.b.) for fried samples obtained after	260
	fat-frying at different temperatures	
Table 6.6	Moisture ratio values for fried samples obtained after fat-	261
	frying at different temperatures	
Table 6.7	Modelling parameters for moisture transfer during deep-	261
	fat frying (DF) of meat analogues at different temperatures	
Table 6.8	Modelling parameters for oil uptake during deep-fat frying	262
	(DF) of meat analogues at different temperatures	
Table 6.9	Kinetic modeling for heat transfer during deep-fat frying	263
	(DF) of meat analogues at different temperatures	
Table 6.10	Two-way ANOVA (with interaction) for Cooking Yield	264
	(CY %) and Cooking Loss (CL %) of fried samples	
Table 6.11	Cooking yield (CY) values for fried samples obtained after	265
	fat-frying at different temperatures	

Table 6.12	Cooking loss (CL) values for fried samples obtained after	266
	fat-frying at different temperatures	
Table 6.13	Cooking yield (CY) and cooking loss (CL) values for fried	266
	reference samples at 160 °C	
Table 6.14	Two-way ANOVA (with interaction) for Hardness (g) and	268
	Springiness of fried samples	
Table 6.15	Hardness values for fried samples obtained after fat-frying	269
	at different temperatures	
Table 6.16	Springiness values for fried samples obtained after fat-	270
	frying at different temperatures	
Table 6.17	Hardness and springiness values for fried reference	271
	samples at 160 °C	
Table 6.18	Colour values (L^* , a^* , b^*) for reference samples obtained	272
	after fat-frying at 160 °C	
Table 6.19	In-vitro protein digestibility (IVPD) values for fried	275
	samples obtained after fat-frying at different temperatures	
Table 6.20	In-vitro protein digestibility (IVPD) values for fried	276
	reference samples obtained after fat-frying at 160 °C	
Table 6.21	Moisture content values (% w.b.) for texturized protein	283
	samples obtained after fat-frying (160 °C) and microwave	
	cooking (MW)	
Table 6.22	Cooking yield (CY) values for texturized protein samples	285
	obtained after fat-frying (160 °C) and microwave cooking	
	(MW)	
Table 6.23	Cooking loss (CL) values for texturized protein samples	286
	obtained after fat-frying (160 °C) and microwave cooking	
	(MW)	
Table 6.24	Hardness values for texturized protein samples obtained	287
	after fat-frying (160 °C) and microwave cooking (MW)	
Table 6.25	Springiness values for texturized protein samples obtained	289
	after fat-frying (160 °C) and microwave cooking (MW)	
Table 6.26	Colour values (L^* , a^* , b^*) for texturized protein samples	291
	obtained after fat-frying (160 °C)	

Table 6.27	Colour values (L^* , a^* , b^*) for texturized protein samples	292
	obtained after microwave cooking (MW)	
Table 6.28	In-vitro protein digestibility (IVPD) values for texturized	293
	protein samples obtained after fat-frying (160 °C) and	
	microwave cooking (MW)	
Table 7.1	An overview of the experimental design for the	312
	preparation of Manila tamarind protein spiral wrap	
Table 7.2	Colour properties of the Manila tamarind protein spiral	324
	wrap sample developed through boiling and autoclaving	
Table 7.3	Cooking properties of the Manila tamarind protein spiral	325
	wrap sample developed through boiling and autoclaving	
Table 7.4	Texture profile parameters of the Manila tamarind protein	327
	spiral wrap sample	
Table 7.5	Physicochemical and functional properties of the Manila	329
	tamarind protein spiral wrap sample	
Table 7.6	Textural properties of the Manila tamarind protein spiral	331
	wrap sample	
Table 7.7	Physico-chemical characteristics and cooking quality of	334
	the fresh, formulated and standard nuggets	
Table 7.8	Texture profile characteristics and colour values (L^* , a^* ,	335
	b^*) of the fresh, formulated and standard nuggets	
Table 7.9	Changes in the characteristics of fresh, formulated and	343
	standard nuggets during frozen storage	
Table 7.10	Colour value parameters of the fresh nugget samples	353
	during 50 days of storage	
Table 7.11	Sensory quality changes of fresh nuggets during 50-day	360
	frozen storage period	
Table 7.12	Total plate count of the fresh, formulated and standard	363
	nugget samples (during 50 days of storage)	
Table 7.13	Yeast and mold count of the fresh, formulated and standard	713
	nugget samples (during 50 days of storage)	

LIST OF FIGURES

Figure No.	Title	Page No.
Figure 2.1	Manila tamarind tree (A) and fruits on its branches (B)	19
Figure 2.2	Manila tamarind fruit pods (A) and seeds (B)	22
Figure 2.3	Different types of meat-analogue products available in the	30
	market	
Figure 2.4	Issues related to the production of animal meat	37
Figure 2.5	Different types of meat analogue products (different forms	39
	and ingredients) available in the market along with their	
	examples	
Figure 2.6	Value Chain Mapping of Plant-Based Meat Analogues	40
Figure 3.1	Cell viability results of the Manila tamarind seed flour extract	89
	(Aqueous and Ethanolic) on HEK-293 cells	
Figure 3.2	Electrophoretic pattern of Manila tamarind seed protein and	113
	modified proteins	
Figure 4.1	An overview of all the steps involved in the preparation of	133
	freeze texturized protein	
Figure 4.2	An overview of step-by-step processes involved in	135
	preparation of freeze structured protein	
Figure 4.3	3D surface plot of (A) Hardness, (B) Springiness, (C) Fibre	144
	content, and (D) Protein content of texturized protein	
Figure 4.4	Visual appearance of the texturized protein prepared	147
	through freeze texturization process	
Figure 4.5	Scanning electron micrographs of the freeze-texturized	167
	protein (sample) at different magnifications	
Figure 4.6	Scanning electron micrographs of the texturized vegetable	167
	protein (reference) at different magnifications	
Figure 4.7	Flowchart depicting the steps used in the formulation of the	169
	texturized proteins prepared through freeze texturization and	
	freeze structuring and comparison of their quality parameters	
	with texturized soy protein (reference sample)	

Figure 4.8	Visual appearance of the texturized proteins prepared through	170
	freeze texturization (A) and freeze structuring (B)	
Figure 5.1	Degree of hydrolysis at various enzyme concentrations (%)	207
	and time (h)	
Figure 5.2	3-D surface plot of sensory score (SS) of the developed	218
	chicken like flavour	
Figure 5.3	Electrophoretic pattern of Manila tamarind seed protein,	224
	modified protein and CPF	
Figure 5.4	Chromatographic and mass spectrometric analyses of CPF	230
	sample (Top panel displays the total absorbance	
	chromatograms (PDA), the middle panel shows the total ion	
	chromatograms (TIC), and the bottom panel presents the mass	
	spectra for the sample	
Figure 6.1	The appearance of the of texturized protein samples before	255
	frying (0 s) and fried samples after deep-fat frying at different	
	frying times (30, 60, 90, 120, 150, 180 s) and temperatures	
	(A-150 °C, B-160 °C, C-170 °C)	
Figure 6.2	The appearance of the reference samples before frying (0 s)	256
	and after deep-fat frying at different frying times (30, 60, 90,	
	120, 150, 180 s) and at a temperature of 160 $^{\circ}\mathrm{C}$	
Figure 6.3	The surface color variations (a: L^* , b: a^* , c: b^*) of the samples	273
	during deep-fat frying at different temperatures (150, 160 &	
	170 °C) and time (0, 30, 60, 90, 120, 150, 180 s)	
Figure 6.4	The total color change ΔE of the samples during deep-fat	274
	frying at different temperatures (150, 160 &170 °C) and time	
	(0, 30, 60, 90, 120, 150, 180 s)	
Figure 6.5	The microstructural changes of samples before (a) and after	277
	(b) frying at 160 °C for 90 s	
Figure 6.6	The microstructural changes of reference samples before (a)	278
	and after (b) frying at 160 °C for 90 s	
Figure 6.7	Correlation heatmap showing the relationships between mass	279
	transfer parameters (k_1, k_2, k_3) , heat transfer parameters $(D_{eff},$	

	α), and product quality attributes (O _{eq} , Hardness, Springiness, and ΔE)	
Figure 6.8	The appearance of the of texturized protein samples before	282
8	cooking (0 s) and cooked samples after frying (a) and MW	
	cooking (b) at different cooking times (30, 60, 90, 120, 150,	
	180 s)	
Figure 6.9	Correlation heatmaps for fat-frying at 160 °C and microwave	296
	cooking, showing the relationships between moisture removal	
	and product quality attributes	
Figure 7.1	Elastic (G') and Viscous (G") moduli of dough samples (a-	322
	solid: liquid of 2:1; b- solid: liquid of 1:1)	
Figure 7.2	Visual appearance of the protein spiral wrap samples after	330
	preparation	
Figure 7.3	Sensory analysis of the formulated and standard nuggets	338
Figure 7.4	Appearance of the nugget samples; fresh nuggets (A);	340
	formulated nuggets after deep fat frying at 160 °C for 90 s (B);	
	standard nuggets* after deep fat frying at 160 °C for 90 s (C)	
Figure 7.5	Effect of storage on hardness of the formulated and standard	344
	nuggets	
Figure 7.6	Effect of storage on springiness of the formulated and	345
	standard nuggets	
Figure 7.7	Effect of storage on cohesiveness of the formulated and	347
Ei 7.0	standard nuggets	240
Figure 7.8	Effect of storage on chewiness of the formulated and standard nuggets	349
Figure 7.9	Effect of storage on lightness value (L^*) of the formulated and	350
J	standard nuggets	
Figure 7.10	Effect of storage on redness/greenness value (a^*) of the	351
	formulated and standard nuggets	
Figure 7.11	The sensory characteristics of standard and formulated	355
	nuggets over a 50-day frozen storage period ((a): appearance,	
	(b): color, (c): flavor, (d): taste, (e): texture, and (f): overall	
	acceptability)	

Figure 7.12	Appearance of the formulated (left side) and standard nuggets	356
	(right side) over a 50-day frozen storage period	
Figure 7.13	Appearance of the fresh nuggets over a 50-day frozen storage	358
	period	
Figure 7.14	Total plate count (Bacteria) of the standard and formulated	262
	nugget samples during 50 days of storage	
Figure 7.15	Yeast and mold count of the fresh, formulated and standard	364
	nugget samples during 50 days of storage	

LIST OF ABBREVIATIONS

 ΔE Colour difference

3-MCPD 3-monochloropropane-1,2-diol

*a** Red-green chromaticity

AA Amino Acids

AAS Amino Acid Score

AC Autoclave

ANOVA Analysis of Variance

AOAC Association of Official Analytical Chemists

*b** Yellow-blue chromaticity

BV Biological Value

CLSM Confocal Laser Scanning Microscopy

CPF Chicken-like processed flavour

Cryo-SEM Cryogenic Scanning Electron Microscopy

DH Degree of hydrolysis

DMA Dynamic Mechanical Analysis

DMSO Dimethyl Sulfoxide

DPPH 2,2-Diphenyl-1-picrylhydrazyl

DSC Differential Scanning Calorimetry

DT Degree of Texturization

DW Distilled water

EA Emulsifying activity

EAA Essential Amino Acids

EAAI Essential Amino Acid Index

ES Emulsifying stability

F_L Parallel cutting force

F_V Vertical cutting force

FTIR Fourier-Transform Infrared Spectroscopy

GCMS Gas Chromatography Mass Spectrometry

HEK-293 Human Embryonic Kidney cell line

HMMA High moisture meat analogues

HPLC High-Performance Liquid Chromatography

IVPD In-vitro Protein Digestibility

IVPDCAAS In-vitro Protein Digestibility Corrected Amino Acid Score

JFF Jackfruit flour
L Liquid fraction

L* Lightness

LCMS Liquid Chromatography Mass Spectrometry

LGC Least Gelation Concentration

MC Moisture Content

MTGase Microbial Transglutaminase

MTSF Manila tamarind seed flour

MTSPI Manila tamarind seed protein isolate

MTT Dimethylthiazol-diphenyltetrazolium-bromide

MW Microwave

NEAA Non-essential amino acids

OAC Oil absorption capacity

OHC Oil holding capacity

PBMA Plant-Based Meat Analogues

PBS Phosphate Buffered Saline

PI Protein Isolate

PS Protein solubility

RSM Response surface methodology

S Salt

SA Sodium Alginate

SDS-PAGE Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

SEM Scanning electron microscopy

SPH Seed protein hydrolysates

TPA Texture profile analysis

TSP Texturized Soy Protein

TVP Texturized Vegetable Protein

US Ultrasound

US30 Ultrasound for 30 min

WAC Water Absorption Capacity

WG Wheat Gluten

WHC Water holding capacity