Development of plant-based meat analogues from Manila tamarind (*Pithecellobium dulce*) seeds

A thesis submitted in part fulfillment of the requirements for the degree of

Doctor of Philosophy

Awanish Singh

Registration No. TZ203926 of 2023

School of Engineering

Department of Food Engineering and Technology

School of Engineering

Tezpur University, Tezpur-784028

Assam, India

October 2025

Chapter-8

Summary, Conclusions and Future Scopes

The increasing global demand for sustainable and ethical protein sources has driven extensive research into plant-based meat alternatives. This thesis explores the potential of Manila tamarind (*Pithecellobium dulce*) seed proteins as a novel ingredient for meat analogue development. The study investigates the physicochemical, functional, and structural properties of Manila tamarind seed proteins in native and modified form, evaluating their suitability for mimicking the texture, taste, and nutritional profile of conventional meat. Comprehensive extraction and characterization processes were employed to optimize protein yield and quality, ensuring compatibility with meat analogue formulations. The results demonstrated that Manila tamarind seed proteins exhibited high solubility, emulsification, oil and water-holding capacities, making them a promising alternative protein source. Additionally, freeze texturization was employed to achieve meat-like fibrous structures. Sensory analysis and nutritional profiling revealed that the developed plant-based meat analogues possess desirable organoleptic properties and a well-balanced amino acid composition, contributing to their feasibility as a functional food. The study concludes that Manila tamarind seed proteins have significant potential in the plant-based meat industry, offering a sustainable, cost-effective, and nutrient-rich alternative to animal-derived proteins. However, challenges such as scale-up production, consumer acceptance, and regulatory approvals need further investigation. Future research should focus on enhancing processing techniques, and fortification with essential micronutrients to improve overall product quality. This study serves as a foundation for further innovations in plant-based protein technologies, contributing to a healthier and more sustainable global food system.

The following points of conclusions obtained from the thesis are summarized below:

- ❖ Manila tamarind seed flour contained 32.59% protein content with protein isolate achieving 85.17% purity after extraction processes.
- ❖ Amino-acid profile demonstrated comparability to soy protein isolate while exhibiting superior sulfur-containing amino acid content for enhanced nutritional value.
- ❖ Cytotoxicity assessment on HEK-293 cells revealed ethanolic extract superior

safety profile with both extracts maintaining >50% cell viability at 500 µg/mL concentrations.

- ❖ Ultrasound (US) treatment significantly ($p<0.05$) enhanced protein solubility, water absorption capacity, oil absorption capacity, emulsifying activity, emulsifying stability, and $L*$, $a*$, $b*$ color parameters.
- ❖ Autoclave (AC) treatment decreased certain functional properties such as solubility, water and oil absorption capacities (WAC and OAC), and emulsifying characteristics when compared with untreated samples.
- ❖ Textural properties including firmness, cohesiveness, and consistency improved substantially with both modification treatments enhancing solution-forming capacity.
- ❖ Least gelation concentration decreased with modification treatments indicating superior gelling ability, with US30 treatment demonstrating optimal gel formation requiring minimal protein quantity.
- ❖ Combined US and AC treatments resulted in overall functional property enhancement primarily attributed to ultrasonic treatment effects.
- ❖ SDS-PAGE analysis revealed that high molecular weight proteins remained resistant to treatments while low molecular weight proteins experienced slight structural modifications.
- ❖ Ultrasound treatment proved highly effective for improving functional properties making the protein suitable for diverse food formulation requirements.
- ❖ The investigation employed freezing technique to create porous arrangements with anisotropic layers for modulating rheological characteristics of Manila tamarind seed protein food gels.
- ❖ Freezing rate and duration significantly impacted structural formation requiring optimization for scalable method development and commercial application.
- ❖ D-optimal mixture design was utilized for optimizing component compositions and identifying appropriate mixture formulations to achieve desired structural goals.
- ❖ Optimized formulation comprised protein isolate (6.53%), wheat gluten (1.23%), jackfruit flour (1.15%), sodium alginate (3.6%), and liquid fraction (87.48%).
- ❖ Both freeze-texturization and freeze-structuring methods were employed for preparing optimized formulations with comparative performance evaluation.
- ❖ Freeze-texturized protein demonstrated superior protein content (64.12% d.b.),

cooking yield (86.33%), and degree of texturization (1.42) compared to freeze-structured alternatives.

- ❖ Freeze-structured protein exhibited more compact and water-retentive matrix characteristics suitable for specific applications requiring moisture retention.
- ❖ Both processing methods produced texturized proteins with properties comparable to or exceeding commercial texturized soy protein reference samples.
- ❖ Physicochemical, functional, and structural properties were comprehensively analysed and benchmarked against commercial standards for validation.
- ❖ Fibrous microstructure analysis revealed promising meat-like texture characteristics suitable for consumer acceptance in plant-based applications.
- ❖ Evaluation of optimized formulation confirmed effectiveness of selected constituents in achieving desired product attributes and performance targets.
- ❖ Research demonstrated significant potential of freeze-texturized proteins as sustainable plant-based alternatives for animal-derived products.
- ❖ The study established Manila tamarind seed protein as viable ingredient for developing nutritionally valuable and environmentally sustainable meat alternatives.
- ❖ The present investigation successfully demonstrated feasibility of transforming Manila tamarind seed protein isolate into viable chicken-like flavouring agent through systematic two-stage processing approach.
- ❖ Enzymatic hydrolysis optimization revealed 0.1% bromelain concentration applied for 6 h at 50 °C yielded most effective protein breakdown with 38% degree of hydrolysis.
- ❖ Flavor precursor selection included glucose and ribose as reducing sugars combined with cysteine, methionine, and glutamic acid as essential amino acids.
- ❖ Response surface methodology identified temperature as critical variable affecting flavor development with optimal conditions at 103 °C for 90 min.
- ❖ Final product exhibited brownish coloration with characteristic meaty, roasted, fatty, and umami flavor notes suitable for food applications.
- ❖ SDS-PAGE analysis revealed significant protein structural modifications during processing confirming effective enzymatic and thermal treatment impacts.
- ❖ GCMS and LCMS analysis identified key volatile compounds including thiols, pyrazines, and Maillard reaction products contributing to authentic chicken-like flavor profile.

- CPF (Chicken-like processed flavour) shows significant potential applications as flavouring agent in plant-based meat products and various food formulations.
- This study examined moisture loss and fat absorption kinetics in texturized protein-based meat alternatives during deep-fat frying at 150 °C, 160 °C, and 170 °C, while comparing quality parameters between conventional frying at 160 °C and microwave cooking at 900W.
- Frying caused progressive darkening and structural modifications, with elevated temperatures accelerating color changes and moisture reduction.
- Moisture content declined significantly during frying, with the most substantial loss occurring in the initial 60 s and fat absorption increased proportionally with frying duration, showing greater uptake at higher processing temperatures.
- Kinetic modelling demonstrated non-linear patterns in both moisture transfer and oil absorption rates, establishing 160 °C as the optimal temperature for moisture management.
- Texture analysis revealed substantial increase in hardness values and minor reductions in springiness throughout the frying process.
- Color evaluation showed consistent decreases in lightness, redness, and yellowness parameters, with cumulative color changes expanding over processing time.
- *In-vitro* protein digestibility enhanced markedly during frying, with superior digestibility achieved at higher processing temperatures.
- Correlation studies revealed intricate relationships connecting mass transfer, heat transfer, and quality characteristics during deep-fat frying operations.
- Comparative assessment of thermal processing techniques established fat-frying at 160 °C as the superior methodology for texturized protein preparation, offering significant benefits in maintaining sensory and physical attributes.
- The investigation concluded that 160 °C fat-frying represented the optimal thermal processing approach for texturized protein products, effectively harmonizing physicochemical stability with nutritional accessibility.
- This study examined the preparation and characterization of Manila tamarind protein spiral wraps and meat analogue nuggets, with particular focus on shelf-life stability during frozen storage conditions.
- The protein spiral wrap dough demonstrated distinct viscoelastic behavior that varied according to solid-to-liquid ratios in the formulation.

- Boiling treatment produced wraps with lighter and more vibrant coloration compared to autoclaving methods.
- The optimal formulation demonstrated high protein content, favorable moisture retention capacity, and desirable textural attributes.
- The formulated nuggets contained lower moisture levels, higher fat content, and slightly reduced protein and mineral concentrations compared to standard nuggets.
- Textural analysis indicated that formulated nuggets exhibited lower hardness, cohesiveness, springiness, and chewiness values.
- Color analysis revealed that formulated nuggets had reduced L^*, a^*, and b^* values, indicating a darker overall appearance.
- Sensory evaluation showed similar profiles between both nugget types, with formulated nuggets receiving slightly superior ratings for color, taste, and flavor characteristics.
- Throughout 50 days of frozen storage, both nugget varieties experienced gradual deterioration in quality parameters including moisture, fat content, pH levels, texture, and sensory properties.
- Textural parameters such as hardness, springiness, cohesiveness, and chewiness declined slightly during storage, with formulated nuggets showing more pronounced reductions.
- L^* and a^* color values increased during the storage period for both product types.
- Microbiological counts remained at low levels throughout the entire storage duration for both products.
- The research emphasized the complex interactions between ingredient composition, physicochemical modifications, and sensory characteristics in plant-based meat alternatives during frozen storage conditions.

Future scopes

- Further exploration of advanced methods of treatment for protein structural modifications, to enhance functional properties of seed proteins for diverse food applications such as extruded products, bakery items, and meat analogues.
- Comprehensive investigation to optimize freezing parameters (rate and duration) to develop robust techniques for creating layered and porous structures in plant-

based protein gels, aiming to establish standardized industrial processing protocols for sustainable protein alternatives.

- ❖ Detailed research on heat and mass transfer mechanisms to support manufacturers in developing high-quality, commercially viable meat alternative products with consistent sensory and nutritional characteristics.
- ❖ The research community must continue to bridge the sensory gap between traditional and alternative protein nuggets, focusing on creating products that not only meet nutritional requirements but also provide a satisfying culinary experience.