ABSTRACT

The growing demand for sustainable and plant-based protein alternatives has driven innovative research into utilizing underutilized agricultural resources for meat substitute development. Manila tamarind (*Pithecellobium dulce*) seeds, traditionally considered agricultural waste, present a promising source of functional proteins that can be transformed into high-quality meat analogues through advanced food processing technologies. This research explores about transforming Manila tamarind seed proteins into chicken-flavoured texturized protein products through innovative processes. The study involved extraction and modification of native proteins from Manila tamarind seeds, employing chemical or physical treatments to enhance their functional properties and structural capabilities. These modified proteins were subsequently processed using advanced texturization techniques like freeze texturization to create fibrous, meat-like structures that effectively mimicked animal protein textures. To achieve authentic chicken-like sensory characteristics, the texturized proteins underwent enzymatic hydrolysis, where bromelain enzyme systematically broke down protein chains to generate essential flavor precursors while simultaneously improving digestibility and bioavailability. This enzymatic treatment was strategically followed by a controlled two-step thermal processing protocol specifically designed to promote Maillard reaction development and generate complex flavor compounds that accurately replicated chicken meat's distinctive taste profile. The research also conducted comprehensive cooking performance evaluations using two distinct heating methodologies, deep-fat frying and microwave heating. The optimized texturized proteins were then formulated into innovative protein spiral wraps and non-breaded nugget products. Extensive storage stability studies were implemented to establish critical shelf-life parameters, systematically monitoring changes in texture, flavor intensity, nutritional composition, and microbial safety across various storage conditions and time periods.

Overall, the research conclusively demonstrated the technical and commercial feasibility of converting underutilized Manila tamarind seed proteins into high-value texturized protein, chicken-like flavour, and texturized protein-based products, positioning them as promising alternatives in the rapidly expanding plant-based meat substitute market while contributing to sustainable protein utilization from agricultural waste streams.

To accomplish the goal, the thesis is divided into 8 chapters as follows:

Chapter 1 provides an overview of the significance of the development of plant-based meat analogues, focusing on the growing global interest in sustainable, health-conscious alternatives to conventional meat. It outlines how meat analogues, designed to mimic the taste, texture, and appearance of animal meat, are becoming increasingly popular due to health concerns, environmental sustainability, and ethical considerations surrounding livestock farming. The chapter also discusses about traditional protein sources like soy and wheat proteins which have long been used for such products, but there's a rising interest in underutilized plant proteins, such as those from the Manila tamarind seeds. These seeds, typically discarded as waste, are nutrient-rich, containing essential amino acids, antioxidants, and bioactive compounds, making them a promising candidate for meat analogue development. The chapter highlights key challenges in protein extraction, flavor replication, and achieving meat-like texture. It emphasizes the importance of flavor development using plant-based sources to mimic complex meat flavors generated through Maillard reactions. Additionally, the text discusses comparative studies between tamarind-based analogues and soy-based products to assess cooking performance, sensory attributes, and consumer acceptance. The research is framed around four main objectives, including protein modification, flavor development, cooking standardization, and product characterization, contributing to the broader goals of creating sustainable, nutritious, and market-viable plant-based meat alternatives. The chapter concludes by outlining the research objectives, focusing on the formulation, characterization, and application of freeze texturized proteins for the food product development.

Chapter 2 provides an extensive literature review on the growing field of plant-based meat analogues, focusing particularly on alternative protein sources such as Manila tamarind seeds. The chapter explains the environmental, ethical, and health motivations behind the shift from animal-based to plant-based proteins. The chapter explores how plant proteins are isolated and modified through techniques like autoclaving and ultrasonication to enhance their functional properties such as solubility, emulsification, gelation, and water-holding capacity. These properties are vital for mimicking the texture and sensory profile of animal meat. Manila tamarind seeds, often discarded, are highlighted as a sustainable and protein-rich resource with high nutritional and bioactive potential.

Texturization methods like extrusion and freeze structuring are discussed for transforming modified plant proteins into fibrous, meat-like products. The role of non-protein ingredients like fats, binders, and natural colorants is also examined in replicating the appearance, flavor, and mouthfeel of meat. Flavor development using enzymatic hydrolysis (e.g., bromelain) and the Maillard reaction is emphasized as crucial for creating authentic meat-like taste. Additionally, the chapter explores the nutritional profiles of meat analogues, noting their benefits such as lower cholesterol, higher fiber, and the inclusion of essential micronutrients. Environmental benefits such as reduced greenhouse gas emissions and water usage are underlined as key advantages. Finally, it discusses the standardization of cooking processes and compares the sensory properties of tamarind-based analogues with traditional soy-based ones, reinforcing the potential of Manila tamarind as a viable meat substitute in sustainable food systems.

Chapter 3 discusses about isolation of proteins from Manila tamarind seeds and their physical modification using ultrasound (US) and autoclave (AC) treatments. This study demonstrated that US and AC treatment changed the physicochemical and functional characteristics of Manila tamarind seed protein isolate (MTSPI) to a significant amount. The properties of emulsifying activity (EA), emulsion stability (ES), protein solubility (PS), water and oil absorption capacity (WAC and OAC) and particle density decreased with the application of AC treatment (EA from 56.47 to 49.68%, ES from 52.54 to 39.52%, PS from 64.48% to 49.5%, WAC from 166.66% to 125.69% and OAC from 171.33% to 120.67%, and particle density from 0.474 to 0.455 g/cm^3), however they increased when ultrasound was applied. Whereas, bulk density and darkness increased with the application of both the US (bulk density from 0.31 to 0.43 g/cm^3, L^* from 69.31 to 60.35) and AC (bulk density from 0.31 to 0.52 g/cm^3, L^* from 69.31 to 48.66) treatment. With the exception of bulk density and particle density, all the other functional properties increased when the US and AC treatments were used in combination (EA from 56.47 to 74.48%, ES from 52.54 to 64.73%, PS from 64.48% to 72.38%, WAC from 166.66% to 189.00% and OAC from 171.33% to 197.66%, firmness from 0.10 to 0.12 N, and LGC from 20 to 10 g/100 g), irrespective of their order and time. As per the electrophoretic pattern of SDS-PAGE, the ultrasound and autoclave treatments caused little to no change on the molecular weights of the protein isolates. The ultrasonic cavitation resulted in decreased particle size and greater exposure of hydrophobic bonds of the proteins as there was a change in their secondary and tertiary structure. The application of moist heat during autoclave treatment

resulted in alteration of the hydrophobicity/hydrophilicity balance and modification of structural integrity by weakening hydrogen bonds and functional groups. Thus, modification in functional properties of MTSPI is beneficial as it can be employed as a potential food ingredient for the development of food products like extruded and bakery products and meat analogues.

Chapter 4 investigates about texturization of modified MTSPI using different texturizing techniques. Freeze-texturization and freeze-structuring were successfully applied to produce layered and porous structures in food gels made from Manila tamarind seed protein. The different ingredients and their composition have different impact on the structures that were produced, and they were optimized using D-optimal mixture design. The formulation containing protein isolate at 6.53%, wheat gluten at 1.23%, jackfruit flour at 1.15%, sodium alginate at 3.6%, and liquid (water/sunflower oil) at 87.48% gave the best results. The results further confirmed that the optimized texturized protein exhibited promising physicochemical and textural characteristics. The developed texturized protein had a substantial protein content (64.12% d.b.), desirable cooking properties, and significant potential for use as a plant-based meat alternative. Furthermore, the presence of phenolics and flavonoids demonstrated its potential health benefits, while the fibrous microstructure suggested a promising meat-like texture. The comparison of freeze texturization, and freeze structuring, revealed freeze texturization as the superior method for producing texturized proteins with higher degree of texturization (1.42 for freeze texturization and 1.06 for freeze structuring), comparable with reference sample soy-meat (1.45). The texturization though different technologies using the optimized formulation upheld the effectiveness of the selected constituents in achieving the desired product attributes and for further food applications.

Chapter 5 discusses about development of chicken-like flavoring compounds from plant-based sources. A chicken-like flavouring agent was produced from Manila tamarind seed protein isolate (modified using ultrasound treatment for 30 min) through enzyme hydrolysis (bromelain enzyme) and thermal treatment. Bromelain showed maximum enzyme activity at 50 °C, which was used for hydrolysis of the Manila tamarind seed protein isolates. Hydrolysis of the protein isolate was conducted at various enzyme concentrations (0-0.2%) and times (0.5-24 h). The degree of hydrolysis increased with increase in enzyme concentration and time, with 0.1% enzyme for 6 h yielding the highest

degree of hydrolysis (38%). Color values of the hydrolysates were not significantly affected by enzyme concentration or time. As the hydrolysate alone lacked authentic chicken flavor, it was then thermally processed with reducing sugars and specific amino acids (glucose, ribose, cysteine, methionine, and glutamic acid) to enhance the chicken-like flavor. Response surface methodology was used to optimize the thermal processing conditions, with temperature having a significant effect on sensory scores. The optimized chicken-like processed flavor (CPF) was produced by thermal processing at 103 °C for 90 min. The CPF had a distinct chicken-like flavor profile and has potential as a natural chicken-like flavouring agent.

Chapter 6 explores about standardization of cooking processes of the developed meat analogues through deep-fat frying (150, 160, and 170 °C) and microwave (MW) cooking. Frying resulted in progressive darkening and structural changes in the samples, with higher temperatures leading to faster color development and moisture loss. Moisture content decreased significantly during frying (73.29 to 23.11% w.b.), with the most rapid loss occurring within the first 60 s. Fat content increased with frying time (14.92 to 35.26% d.b.), with higher temperatures resulting in greater fat absorption. Kinetic modelling revealed non-linear trends in moisture transfer and oil uptake rates, with 160°C identified as the optimal temperature for moisture transfer ($k_1 = 6.4\times10^{-3}s^{-1}$, $D_{eff} = 2.67\times10^{-7}m^2/s$). Cooking yield decreased (from 100 to 50.97% at 170 ℃) and cooking loss increased (from 0 to 49.03% at 170 ℃) during deep-fat frying, with more pronounced changes at higher temperatures. Textural analysis showed significant increases in hardness (614.9 g to 1908.02% at 160 ℃ and 120 s) and slight decreases in springiness (0.976 to 0.895 at 170 ℃ and 180 s) during frying. Color analysis demonstrated a consistent decline in lightness (51.01 to 32.25), redness (7.6 to 1.03), and yellowness (12.1 to -0.23) values, with total color change increasing over frying time. *In-vitro* protein digestibility improved significantly during frying (from 64.33 to 84.98%), with higher temperatures resulting in greater digestibility. SEM analysis revealed microstructural changes, including increased porosity and heterogeneity, after frying. Correlation analysis highlighted complex relationships between mass transfer, heat transfer, and product quality parameters during the deep-fat frying process. Based on the comparative evaluation of thermal processing methods, fat-frying at 160 °C emerged as the superior approach for cooking texturized protein products, demonstrating significant advantages in preserving organoleptic and physical properties.

Chapter 7 discusses about the preparation and characterization of Manila tamarind protein spiral wraps and meat analogue nuggets (with emphasis on shelf-life stability under frozen storage conditions). The protein spiral wrap dough exhibited distinct viscoelastic responses based on solid-to-liquid ratios with G' value of 119,140 Pa and G'' values of around 48,209 Pa for solid to liquid ratio of 2:1 and G' value of 75,213 Pa and G'' values of around 33,867 Pa for solid to liquid ratio of 1:1, respectively. Boiling resulted in lighter and more vibrant colored wraps compared to autoclaving. Autoclaving yielded higher cooking yields (191.46%, 2:1 for 15 min), while boiling produced superior textural properties (hardness of 458.08 g, 2:1 for 15 min). The best formulation had high protein content (60.28% d.b.), favorable moisture retention (expressible moisture of 43.66%), and good textural attributes (hardness of 428.07 g, springiness of 54.56%, guminess of 94.31 g). The formulated nuggets had lower moisture content (28.66%), higher fat content (17.95%), slightly lower protein (27.20%) and mineral content (2.42%) compared to standard nuggets (moisture content of 30.82%, fat content of 14.55%, protein content of 28.46%, and ash content of 2.68%). Cooking quality of the formulated nuggets (cooking yield of 92.56%) was comparable with that of the standard nuggets (cooking yield of 94.91%). Textural analysis revealed lower hardness (1450.46 g), cohesiveness (0.35), springiness (0.74) and chewiness (375.67 g.s) in formulated nuggets. Color analysis showed formulated nuggets had slightly lower L^* (50.55), a^* (6.60) and b^* (9.15) values indicating darker appearance and better colour development due to Mailard browning. Sensory evaluation demonstrated similar profiles for both nuggets (7.5 for standard and 7.4 for formulated), with formulated nuggets having slightly better color (7.2), taste (7.3) and flavor scores (7.6). During 50 days of frozen storage, fresh, formulated as well as standard nugget types showed minimal to no decline in quality parameters like moisture (28.66% at day 0 to 28.15% at 50 day), fat (17.95% at day 0 to 17.46% at 50 day), pH (6.54 at day 0 to 6.48 g at 50 day), appearance and sensory attributes (7.6 over 50-day period). Textural parameters like hardness, springiness, cohesiveness and chewiness remained almost same over the 50 day storage period. L^* values increased during storage with L^* value changing from 50.55 to 53.82 and a^* values reduced a bit with values changing from 6.60 to 6.30. Sensory attributes remained almost same, with formulated nuggets maintaining slightly better color and flavor stability and a liitle bit of textural degradation due to thawing. Microbiological counts remained nil throughout the storage period. The study highlighted the complex interplay of ingredient composition,

physicochemical changes and sensory attributes in development of plant-based meat alternative products and their effect on the product characteristics during frozen storage.

Chapter 8 summarizes the key findings, conclusions, and future directions of the research on plant-based meat analogues developed using protein isolated from seeds of Manila tamarind. The research successfully modified the Manila tamarind seed proteins using physical treatments to enhance their functional properties, such as solubility, water-holding capacity, and gelling ability, key factors in achieving meat-like texture. These modified proteins were then texturized using freeze texturization and freeze structuring to form fibrous structures that mimic animal meat. To replicate the savory flavor and improve digestibility, the modified proteins underwent enzymatic hydrolysis, where specific enzymes broke down protein chains to release flavor precursors. This was followed by a two-step thermal process to induce the Maillard reaction, producing complex flavor compounds and browning effects characteristic of cooked chicken. The resulting product's cooking performance was tested using deep-fat frying and microwave heating to evaluate oil absorption, texture development, and browning behavior. These insights guided the development of protein-spiral wraps and non-breaded nuggets, eliminating the need for conventional coatings while maintaining sensory appeal and structural stability. To assess commercial potential, storage stability studies were conducted under various conditions to monitor changes in texture, flavor, nutritional value, and microbial safety over time. The study effectively demonstrated the potential of Manila tamarind seed proteins as a sustainable, functional, and flavorful ingredient in the growing plant-based meat market, providing a promising alternative to traditional soy-based products.

Keywords: Autoclaving, cooking quality, enzymatic-hydrolysis, freeze-texturization, gelling ability, protein-spiral wraps, ultrasonication