Chapter-5

Development of plant-derived flavoring components to mimic the flavor of animal meat

5.1.Introduction

Thermally processed flavourings have become widely used as savoury flavouring agents in various products, such as soups, sauces, snacks, and ready meals. The meaty or beefy flavour is generated by heating a combination of protein hydrolysate, amino acids, and reducing sugars under controlled conditions through the Maillard reaction (Manley & Ahmedi, 1995). Thermal processing of various plant protein sources such as soy (Wu & Cadwallader, 2002), wheat (Lee et al., 2012), and *Brassica* (Guo et al., 2009) has produced meaty or beefy flavours to replace flavouring agents from animal protein sources. Protein hydrolysates from various plant sources have generally been used to produce meat-like flavourings (Farid et al., 2024). A high flavour quality is difficult to ensure for chicken-like flavouring that is produced from animal sources because of their high cost and the challenge of maintaining consistent flavor profiles (Imm & Lee, 1999).

For centuries, Manila tamarind (*Pithecellobium dulce*) has been used in traditional cooking due to its versatile properties. Manila tamarind seeds, often discarded as waste products, are good sources of plant protein and contain taste-active amino acids, such as aspartic acid, glutamic acid, arginine, and lysine, in addition to a low-fat content. Manila tamarind is one of the widespread legumes that is cultivated in tropical countries. In many parts of Asia, Manila tamarind seeds are often discarded after consuming the fruit pulp. In human diets, the consumption of raw Manila tamarind seeds does not provide optimal nutritional value, due to the presence of anti-nutritional factors (Normah, & Noorasma, 2018). After processing Manila tamarind seeds, the protein isolate is rich in proteins, which contain high amounts of essential amino acids. However, the potential peptides and amino acids in various plant sources, including Manila tamarind seeds, are only partially digested using gastrointestinal enzymes, and they are lost during the cooking process (Sarmadi & Ismail, 2010). The production of potential peptides of Manila tamarind seed protein by human gastrointestinal digestion is still limited, due to the complex protein structure with low digestibility and poor solubility at acidic pH (Sá

et al., 2020), and the uncontrolled hydrolysis of gastrointestinal enzymes (Catalano et al., 2024).

An effective process to improve the physicochemical characteristics, volatile compounds, and organoleptic quality of plant proteins is selective enzymatic hydrolysis under controlled conditions, which generates available peptides and amino acids with less salt and carcinogenic compounds, such as mono- and dichloropropanols or 3-monochloropropane-1,2-diol (3-MCPD), than acid hydrolysis (Sun et al., 2022; Qiang et al., 2023). The application of hydrolytic enzymes to hydrolyse plant protein can enhance the flavour of the protein hydrolysate because it increases the quantity of amino acids and low molecular weight peptides that possess unique taste properties, including sweet, salty, sour, bitter, and umami tastes (Peighambardoust et al., 2021; Fan et al., 2022; Farid et al., 2024). The types of proteases and protein substrate affect the functional properties and flavour profile of the protein hydrolysate (Cheng et al., 2024).

Various commercial proteases are employed in enzymatic hydrolysis, one of which is bromelain. Stem bromelain (EC 3.4.22.32), which is a cysteine endoproteinase, is extracted from the core of the stem of pineapple fruit (*Ananas comosus*), which is a common and abundant plant grown in Thailand. Bromelain is a potential enzyme for the production of enzymatic protein hydrolysates and is widely used in various food products because of its broad specificity in the cleavage of peptide bonds and its stable activity over a wide pH range (pH 4.0–8.0). Although many studies have reported the functional properties (antioxidant and bioactive capacities) and flavour of plant protein hydrolysates produced using bromelain hydrolysis (Shahbal et al., 2023; Gasparre et al., 2025), a hydrolysate from Manila tamarind seed protein isolate using bromelain to generate chicken-like flavour has not been characterised.

Nevertheless, the flavour of plant protein hydrolysates does not necessarily impart a chicken-like flavouring. Therefore, thermal processing may improve the taste and odour of the protein hydrolysates (Lin et al., 2024). The production of chicken-like flavour from thermally processed enzymatic hydrolysates of Manila tamarind seed protein requires investigation. Therefore, the objectives of this study were to produce a protein hydrolysate from Manila tamarind seed protein isolate using bromelain and to characterise the physicochemical properties and volatile components of this protein hydrolysate followed by a sensory evaluation. The enzymatic bromelain Manila tamarind

seed protein hydrolysate (SPH) was used as the precursor of a thermally processed chicken-like flavouring agent. Moreover, response surface methodology (RSM) was applied to evaluate the optimal hydrolysis conditions.

5.2.Materials and methods

5.2.1. Materials and reagents

Manila tamarind seed protein isolates (US30- ultrasound for 30 min) obtained from Objective 1 were used as the base material for the study on flavour development. Other chemicals and reagents of analytical grade like Potassium phosphate dibasic trihydrate, hydrochloric acid (HCl), casein, sodium hydroxide (NaOH), trichloroacetic acid, Folin & Ciocaltea's phenol reagent (Folin's phenol reagent), sodium carbonate (anhydrous), sodium acetate, calcium acetate, L-tyrosine, petroleum ether, Bovine Serum Albumin (BSA), ninhydrin and Bradford reagent, phosphate buffer, and Bromelain utilized in analysis were purchased from Octagon Chemicals & Instruments, Zenith India, Guwahati, Assam, India.

5.2.2. Bromelain enzyme activity assay

Enzyme activity assay was performed using the modified method of Cupp-Enyard (2008) at temperatures of 40, 50, and 60 ℃ using a series of steps. A 0.65% casein solution was taken and equilibrated in a water bath for approximately 5 min. Enzyme solutions of varying concentrations, specifically bromelain enzyme (EC-3.4.22.32), were then added to the casein solution and incubated for 10 min. Following this, a 110 mM trichloroacetic acid solution was introduced, and the mixture was further incubated for 30 min. Tyrosine standards were prepared in the concentration range of 0.05 to 0.5 μM. The solutions were subsequently filtered and mixed with reagents. The absorbance of both the samples and the standard was measured using a spectrophotometer at a wavelength of 660 nm. Finally, the data points from the standard were used to calculate the enzyme activity values of the test sample. The temperature corresponding to highest enzymatic activity was selected for further enzymatic hydrolysis for flavour development purposes (Liliany et al., 2018).

5.2.3. Preparation of the liquid protein hydrolysates

Manila tamarind seed protein isolate (US30) was dispersed in sterile water at a concentration of 5 g/100 mL. The dispersions were then pre-incubated at 50 ℃ for 10 min to ensure optimal bromelain activity. The bromelain enzyme was added at various concentrations of enzyme/ substrate ratios (E/S) of 0%, 0.05%, 0.1%, 0.15%, and 0.2% (weight of enzyme/weight of protein isolate). The hydrolysis reaction was conducted at a temperature of 50 °C for varying time intervals of 0.5, 3, 6, 12, 18, and 24 h. After the hydrolysis period, the reaction was terminated by heating the mixture to 95 °C for 15 min. The enzyme hydrolysates were then centrifuged at 5000g for 15 min at 25 °C. The supernatants were filtered through Whatman No. 1 filter paper and collected in amber coloured glass bottles at -20 ℃ prior for further analysis (Nurdiani et al., 2024).

5.2.4. Determination of the physicochemical properties of the liquid seed protein hydrolysate (SPH)

The physicochemical properties (colour, and degree of hydrolysis) of the liquid SPHs that were produced under all conditions were characterized.

5.2.4.1.Measurement of colour parameters

The colour of liquid seed protein hydrolysate (SPH) samples was measured using a colorimeter (MiniScan® EZ; HunterLab, Reston, VA) calibrated with white and black standard plates. 30 mL of each sample was placed in a glass cuvette, and the L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) values were recorded (Selamassakul et al., 2018).

5.2.4.2.Measurement of degree of hydrolysis

The degree of hydrolysis (DH) was determined using the method described by Sathivel et al. (2003). The percentage of DH is defined as the percentage of soluble protein in trichloroacetic acid (TCA). To perform this analysis, a sample aliquot of 10 mL was mixed with 10 mL of 20% TCA and subsequently centrifuged at 5000g for 15 min at 25 °C. The soluble nitrogen in the supernatant, along with the total nitrogen, was quantified using the Kjeldahl method. The degree of hydrolysis was then calculated using the following equation:

$$\% \text{ DH} = \frac{20\% \text{ TCA Soluble N}}{\text{Total N}} \times 100 \quad (5.1)$$

where Total N represents the total amount of nitrogen in the hydrolysed protein solution, and 20% TCA Soluble N denotes the amount of soluble protein in TCA.

5.2.4.3.Determination of the amino acid composition

The amino acid composition of the Manila tamarind seed protein hydrolysate (SPH) obtained from best enzymatic hydrolysis conditions was evaluated using high-performance liquid chromatography (HPLC) and compared with amino-acid composition of Manila tamarind seed protein isolate (MTSPI), in accordance with the methodology of Wang et al. (2016) with some modifications. Prior to the determination of the amino acid composition, the MTSPI powder was treated with 6N HCl at 110 °C for 24 h. The amino acid content of the SPH was assessed without acid hydrolysis. The acid and enzyme hydrolysed samples were subsequently analysed using HPLC connected to a fluorescence detector, with excitation and emission wavelengths configured at 250 and 395 nm, respectively. The analysis employed a Hypersil GOLD column (4.6 mm×100 mm, 5 μm), which was eluted at a flow rate of 1 mL/min in gradient mode using a combination of eluent A (sodium acetate buffer; pH 4.90) and eluent B (60% acetonitrile). Tryptophan was analysed using high-performance liquid chromatography (HPLC) following basic hydrolysis. Amino acids were quantified after derivatization with diethyl-ethoxymethylenemalonate, using D, L-α-aminobutyric acid as the internal standard and was documented as milligrams (mg) of amino acid per 100 g of protein in the sample solution.

5.2.4.4.Electrophoretic pattern by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protocol of Biswas and Sit (2020) was used to study the electrophoretic patterns of the seed protein hydrolysates (SPH) obtained from best enzymatic hydrolysis conditions. Under reduced conditions, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the electrophoretic pattern of the SPH solution. Centrifugation of the SPHs was performed at 4500 g at 20 ℃ for 20 min. The SPHs were dissolved in the SDS buffer (0.5 M Tris, 2.0% SDS, 0.05% b-mercaptoethanol, pH 6.8) (sample: sample buffer 50 mg:150 μL), heating to 95–100 ℃ for 15 min and again mixed using vortex for some time. SDS buffer was also added to the SPH solution as mentioned

above. SDS-PAGE was confirmed by loading 15 µL of samples onto the gels which were earlier made on a Mini-PROTEAN II system (Bio-Rad). The resolving and stacking gels were made with 15.0% and 10.0% acrylamide, respectively. A Coomassie Brilliant Blue stain was applied to the gel after 45 min of running, followed by 45 min of shaking using water, methanol, and acetic acid in a 45:45:10 ratio. The gels were de-stained with a solution which included acetic acid, methanol, and water in the similar proportion of 45:45:10, washing them with running water, maintained in water overnight, along with scanning them utilizing a Gel Doc system (Bio-Rad Chemi-Doc XRS+).

5.2.5. Experimental design for optimization using response surface methodology (RSM)

A central composite design (CCD) was employed to study the effects of two independent variables: thermal processing time (X_1: 30-150 min) and temperature (X_2: 80-120 °C) on the sensory score (dependent variable) of the developed chicken-like flavor.

Table 5.1: List of all the independent variables with their different combinations

		Factors	
Std	**Run**	**A: Time (min)**	**B: Temperature (°C)**
1	8	47.5736 ~ 47.5	85.8579 ~ 86
2	2	132.426 ~ 132.5	85.8579 ~ 86
3	5	47.5736 ~ 47.5	114.142 ~ 114
4	12	132.426 ~ 132.5	114.142 ~ 114
5	10	30	100
6	7	150	100
7	13	90	80
8	11	90	120
9	4	90	100
10	1	90	100
11	3	90	100
12	9	90	100
13	6	90	100

The experimental design consisted of 13 experimental points **(Table 5.1)** including 5 replicates at the center point. The coded levels for the independent variables were:

- Time (X_1): -1.414 (30 min), -1 (47.57 min), 0 (90 min), +1 (132.43 min), +1.414 (150 min)

- Temperature (X_2): -1.414 (80 ℃), -1 (85.86 ℃), 0 (100 ℃), +1 (114.14 ℃), +1.414 (120 ℃)

5.2.6. Preparation of chicken-like processed flavour

The liquid seed protein hydrolysate (SPH) generated from the optimal hydrolysis conditions was further processed to create chicken-like processed flavour (CPF). The thermal treatment of liquid SPH was conducted following a modified procedure of Manley & Ahmedi (1995). A combination of glucose, ribose, cysteine, glutamic acid, alanine, glycine, and liquid SPH at 2:1:2:2:2:8:15 g was dissolved in 100 mL of distilled water. The solution was subsequently heated as per the temperature and time of the experimental design. Following the reaction, the thermal reaction products were cooled to room temperature and stored at -18 ℃ for further uses. The CPF was evaluated for colour, volatile compounds, and sensory profile.

5.2.7. Sensory evaluation of the chicken-like processed flavour (CPF)

Sensory profiling was conducted on the chicken-like processed flavour (CPF) using the modified methodology of Sun et al. (2023) utilizing quantitative descriptive analysis to check whether chicken like flavour was developed/achieved or not. The intensity of each attribute was evaluated on a scale ranging from 0 to 9 by 15 semi-trained panellists comprising university students (males and females aged between 22 and 32 years old). All semi-trained panellists (university students) had previous experience in descriptive sensory analysis of various food samples. All ingredients used in the preparation were from plant materials and only those panellists were selected who generally consume chicken and chicken used for the preparation of broth was procured from market. The attributes encompassed four odours including meaty, roasted chicken, fatty, and caramel, along with four tastes: umami, sweet, salty, and bitter. The CPF samples (5 mL) were maintained at temperatures in between 80-85 ℃ and presented in opaque disposable plastic cups.

5.2.8. Optimization of processing parameters for development of processed chicken-like flavor

Using Design Expert software, the desired outcomes for each variable and responses were chosen, and the numerical values of the independent variables and simultaneous

optimization of the responses were carried out. For the optimization of the process, the independent parameters time and temperature were kept in range (47-132 min and 85-114 ℃) while the dependent parameter sensory score was kept at maximum so as to obtain product with best flavor qualities. To achieve the optimal solution for the response variable, individual goals were combined into a composite function known as the desirability function. This objective function reflects a desirable value ranging from zero to one. One optimal solution (Time - 90 min, Temperature - 103 ℃ and Sensory Score - 7.43) to this response variable was found with a desirability value of 0.98.

5.2.9. Analysis of the properties of optimized chicken-like processed flavour (CPF)

5.2.9.1.Color measurement

Colour measurements of the optimized chicken-like processed flavour (CPF) were determined by examining approximately 30 mL of the CPF using a colorimeter (MiniScan_ EZ; HunterLab, Reston, VA). The *L**, *a**, and *b** values were measured to quantify the colour intensity or colour saturation (Selamassakul et al., 2018).

5.2.9.2.Sensory evaluation

As per the methodology described earlier in section **5.2.7**.

5.2.9.3.Electrophoretic pattern by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protocol of Biswas and Sit (2020) was used to study the electrophoretic pattern and molecular weight of the chicken-like processed flavour (CPF). Under reduced conditions, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the electrophoretic pattern of the protein isolate solution. The solution was freshly prepared by mixing the CPF in distilled water with the help of a stirrer. After that, centrifugation was performed at 4500 g at 20 ℃ for 20 min. The CPF was dissolved in the SDS buffer (0.5 M Tris, 2.0% SDS, 0.05% b-mercaptoethanol, pH 6.8) (sample: sample buffer 50 mg:150 µL), heating to 95–100 ℃ for 15 min and again mixed using vortex for some time. SDS buffer was also added to the CPF solution as mentioned above. SDS-PAGE was confirmed by loading 15 µL of samples onto the gels which were earlier made on a Mini-PROTEAN II system (Bio-Rad). The resolving and

stacking gels were made with 15.0% and 10.0% acrylamide, respectively. A Coomassie Brilliant Blue stain was applied to the gel after 45 min of running, followed by 45 min of shaking using water, methanol, and acetic acid in a 45:45:10 ratio. The gels were de-stained with a solution which included acetic acid, methanol, and water in the similar proportion of 45:45:10, washing them with running water, maintained in water overnight, along with scanning them utilizing a Gel Doc system (Bio-Rad Chemi-Doc XRS+).

5.2.9.4.Determination of the volatile compound composition using GC-MS

The composition of volatile compounds in the chicken-like processed flavour (CPF) was examined using a headspace solid phase microextraction gas chromatography mass spectrometry (HS SPME-GC–MS) system (789DA MS, Agilent Technologies, USA) (Sonklin et al., 2011). Each sample (3 mL) was transferred into a 20 mL vial and heated at 90 ℃ for 10 min in a GC–MS heating block for headspace analysis. Volatile compounds were adsorbed onto an SPME fibre (50/30 μm) for 20 min. Following equilibrium, the SPME fibre underwent desorption into the injector port at 240 ℃ for 20 min, with the injector operating in split less mode. Helium served as the carrier gas at a constant velocity of 1.0 mL/min. Volatile compounds were separated utilizing a DB-Wax capillary column (30 m×0.25 mm, 0.25 μm film thickness). The oven temperature program proceeded as follows: initial temperature of 55 ℃; elevated to 180 ℃ at 5 ℃/min; further increased to 200 ℃ at 8 ℃/min; and maintained at 200 ℃ for 10 min. Volatile compounds were detected via MSD (scan range of m/z 35–350) at 230 ℃. The identification of compounds relied on comparing their retention time and mass spectrum with data in the Wiley 275 and NIST libraries at a quality match exceeding 85%. A series of n-alkanes (C_8-C_{20}) underwent analysis by direct injection on the GC-MS to obtain retention index (RI) values. The RI data were then compared with previously published literature values.

5.2.9.5.Determination of the volatile compound composition using LCMS

The identification of volatile compounds in the chicken-like processed flavour (CPF) was performed through the modified protocol of Wei et al. (2022) using liquid chromatography-mass spectrum (LC-MS) (Agilent 1290 LC & 6460C Triple Quad MS; Agilent Technologies, USA). The optimized program conditions for the LC-MS were as follows. A reversed-phase Poroshell 120 EC-C18 column (2.1 mm × 100 mm; 4 μm) was

employed and the mobile phases consisted of 3% formic acid in water (A) and 90 % acetonitrile, 5% methanol, and 5% water (B) at a flow rate of 0.35 mL/min. The injection volume measured 3 µL and the column temperature was maintained at 35 °C. The gradient program consisted of: 0–1.5 min, 95% A; 8 min, 85% A; 9.5 min, 80% A; 11.5 min 80% A; 14 min 78% A; 20 min, 72% A; 24 min, 70% A; 29 min, 10% A; 32 min, 10% A; 35 min, 95% A. The MS program was configured as follows: negative ion mode; capillary voltage, 3.5 kV; gas temperature at 250 °C; sheath gas temperature at 280 °C; nebulizer gas at 45 psi; gas flow at 10 L/min and sheath gas flow at 11 L/min. Data acquisition was conducted using mass spectrometry, where volatile compounds were identified according to their retention time and mass spectral data through comparison with known standards and spectral libraries.

5.2.10. Statistical analysis

Statistical analysis was performed with IMS SPSS Statistics 26 software. All measurements were conducted in triplicate, with results expressed as mean ± standard deviation. Statistical differences between samples were evaluated through one-way analysis of variance (ANOVA), followed by Duncan's multiple range test, with $p<0.05$ considered statistically significant.

5.3.Results and discussion

5.3.1. Bromelain enzyme activity at various temperatures

In this study, enzyme activity measurements were conducted to identify the optimal temperature (for use in the hydrolysis process) at which bromelain enzyme exhibited maximum activity. The results revealed that bromelain demonstrated peak activity at 50 ℃. As illustrated in **Table 5.2**, bromelain activity increased significantly ($p<0.05$) with increasing temperature until reaching 50 ℃, after which a declining was observed. This pattern aligns with findings from previous research, which indicated that activity increased as temperature rose from 30 ℃ to 50 ℃, followed by a subsequent decrease at elevated temperatures (Sangkharak et al., 2016; Utami et al., 2019). The maximum enzyme activity for crude bromelain was determined to be at 50 ℃, which consequently facilitated a high degree of hydrolysis (Masri, 2013; Laohakunjit et al., 2017). The findings from this investigation demonstrated that the bromelain enzyme conditions associated with peak enzyme activity occurred at a temperature of 50 ℃, resulting in an

enzyme activity measurement of 0.21 U/mL. To elaborate further, the temperature-dependent activity profile of bromelain observed in this study reflects the fundamental principles of enzyme kinetics. As temperature increases from lower values, molecular collisions between the enzyme and substrate become more frequent, accelerating the reaction rate according to the Arrhenius equation (Whitaker, 2002). This explains the initial rise in bromelain activity with increasing temperature.

Table 5.2: Bromelain enzyme activity at various temperatures

Temperature (℃)	Enzyme Activity (U/mL)
40	0.132 ± 0.001^{c}
50	0.401 ± 0.03^{a}
60	0.185 ± 0.01^{b}

Values are expressed as the average of triplicates ± standard deviation. Different lowercase letter superscripts within the same column, indicate statistically significant differences ($p<0.05$)

The optimal temperature of 50 ℃ represents the point at which the enzyme achieves maximum catalytic efficiency, where the reaction rate is optimized without compromising the structural integrity of the enzyme (Daniel & Danson, 2010). The subsequent decline in enzyme activity above 50 ℃ can be attributed to thermal denaturation of the bromelain protein structure (Jutamongkon & Charoenrein, 2010). At temperatures exceeding the optimum, the increased thermal energy disrupts the non-covalent interactions (hydrogen bonds, ionic interactions, and hydrophobic forces) that maintain the enzyme's three-dimensional conformation, particularly affecting the active site geometry (Bommarius & Paye, 2013). This denaturation progressively reduces the enzyme's ability to bind with substrates and catalyse reactions effectively. This quantification of enzyme activity provides a standardized measure for ensuring consistency in subsequent hydrolysis reactions of the Manila tamarind seed protein isolates (US30), allowing for reproducible degrees of hydrolysis and ultimately affecting the functional properties of the resulting hydrolysates (Kristinsson & Rasco, 2000; Choonpicharn et al., 2015). Subsequently, enzymatic hydrolysis of Manila tamarind seed protein isolates (US30) was performed under these established conditions.

5.3.2. Hydrolysis of Manila tamarind seed protein isolates (US30) using various concentrations of bromelain enzyme and time

5.3.2.1. Analysis of Manila tamarind seeds as a protein source for hydrolysate production

Manila tamarind seeds emerge as an exceptional raw material for protein hydrolysate production due to their outstanding nutritional profile and practical advantages. These seeds contain remarkably high protein levels, reaching up to 40 % of their total composition (Hamdi et al., 2023). Their fat content remains relatively low compared to other protein sources. This favourable high protein ratio creates ideal conditions for efficient protein extraction processes (Hou et al., 2022). The amino acid composition of Manila tamarind seeds demonstrated exceptional balance and quality. Essential amino acids like lysine, leucine, and phenylalanine were present in significant quantities. These particular amino acids are frequently deficient in cereal-based dietary systems (Rao, 2013). The complete amino acid profile makes these seeds valuable for addressing nutritional gaps in various populations. Bromelain enzyme was specifically chosen for the hydrolysis process due to its compatibility with this protein source. The enzyme effectively breaks down the seed proteins while maintaining their beneficial properties. Agricultural abundance represents another crucial factor in selecting Manila tamarind seeds. These seeds are widely available as by-products across tropical regions worldwide. Southeast Asia, India, and parts of Latin America produce substantial quantities annually (Murugesan et al., 2019). This availability ensures consistent supply chains for industrial applications. The transformation potential of these high-protein seeds addresses both nutritional and commercial objectives simultaneously (Rao, 2013). Protein hydrolysates derived from Manila tamarind seeds exhibit multiple functional properties that enhance their utility. Enhanced solubility allows better incorporation into various food systems. Improved emulsification capacity supports applications in processed foods and beverages. Superior foaming stability proves valuable for bakery and confectionery applications (Benjakul et al., 2014; Sonklin et al., 2020). These functional properties remain stable across wide pH ranges, expanding potential applications. Bioactive characteristics add significant value to these protein hydrolysates beyond basic nutrition. Antioxidant properties help prevent oxidative damage in biological systems. Antihypertensive effects support cardiovascular health maintenance. Antimicrobial activities provide natural preservation benefits in food applications

(López-Barrios et al., 2014). These bioactive features make the hydrolysates suitable for functional foods and nutraceutical formulations. Enzymatic hydrolysis using bromelain proves superior to chemical alternatives for several reasons. The process optimizes bioactive peptide release while preserving nutritional integrity. Chemical methods often damage sensitive compounds and reduce biological activity. Enzymatic processes operate under milder conditions that maintain protein quality (Hamdi et al., 2023). The controlled hydrolysis also allows for better customization of final product characteristics based on intended applications.

5.3.3. Physicochemical properties of the SPH

5.3.3.1.Colour parameters of the SPH

The differential colorimetric evaluation (DE) values of liquid enzymatically-produced Manila tamarind seed protein hydrolysates (SPHs) were calculated based on the *L**, *a**, and *b** colorimetric parameters. Comprehensive statistical analysis revealed that neither enzyme concentration nor hydrolysis duration significantly influenced the DE values of the liquid SPHs ($p<0.05$). The measured DE values ranged from 1.92 to 4.03, indicating minimal color variation across different processing conditions. These findings demonstrate that the chromatic characteristics of the liquid SPHs closely resembled those of the control hydrolysate produced through non-enzymatic hydrolysis methods (Benjakul et al., 2014). The liquid SPHs exhibited a distinctive light yellow-green appearance, attributable to the presence of natural pigments inherent to the seed material. Since sensory perception of food products is substantially influenced by their visual characteristics, the specific coloration of the SPH could serve as a valuable indicator of its applicability as a flavouring agent in various food formulations (Spence, 2015).

5.3.3.1.1. Analysis of *L** value of the seed protein hydrolysates

The *L** value is crucial in assessing protein hydrolysates, with lower values indicating darker coloration. **Table 5.4** presents *L** values for seed protein hydrolysates at different concentrations (0–0.2%) over various hydrolysis periods (0.5–24 h). Two-way ANOVA **(Table 5.3)** revealed that enzyme concentration and hydrolysis time both significantly ($p<0.05$) influenced *L** values. There was also a significant ($p<0.05$) interaction effect, since at 0% enzyme, *L** slowly reduced from 28.72 at 0.5 h to 27.22 at 24 h, while at 0.05% enzyme, *L** plummeted down to 24.53 at 6 h before slowly increasing up to 27.14

at 24 h. Also, at 0.1% enzyme, the minimum L^* (23.86) occurred at 6 h and then increased to 26.27 at 24 h. The control sample (0% enzyme concentration) demonstrated relatively stable lightness characteristics throughout the 24 h hydrolysis period. The L^* value decreased gradually from an initial 28.72 to a final 27.22 over the complete duration. This represented a modest 5.2% reduction in lightness over the entire experimental timeframe. The minimal color changes observed in the control sample suggest that enzymatic activity is the primary driver of color modifications during hydrolysis (Ashaolu et al., 2023). Enzyme-treated samples exhibited significantly more dynamic and complex color shifts compared to the control. At 0.05% enzyme concentration, the L^* value dropped significantly ($p<0.05$) from 26.94 to 24.53 by the 6 h mark. This represented an 8.9% reduction in lightness during the initial hydrolysis phase. However, the L^* value subsequently rose to 27.14 at the 24 h endpoint. This pattern showed initial darkening followed by lightening as the hydrolysis process progressed (Wang et al., 2023). The 0.1% enzyme concentration exhibited the most pronounced and dramatic darkening effects among all tested concentrations. The L^* value reached its lowest point of 23.86 at the 6 h mark. This represented a substantial 10.1% reduction from the initial lightness value. The significant ($p<0.05$) darkening likely resulted from protein degradation processes and concurrent Maillard reactions occurring during hydrolysis (Li et al., 2013). Higher enzyme concentrations of 0.15% and 0.2% showed comparatively less darkening than the 0.1% concentration. The 0.15% concentration reached its minimum L^* value of 24.95 at the 12 h mark. The 0.2% concentration achieved its lowest L^* value of 25.56 at the 3 h point. These results suggest that substrate limitations or enzyme inhibition effects occur at elevated enzyme concentrations (Zhang et al., 2024). An interesting inverse relationship emerged between initial L^* values and enzyme concentration across all samples. The control sample maintained the highest initial L^* value at 28.72. This value decreased progressively to 26.19 at the 0.2% enzyme concentration. However, beyond the 0.1% concentration threshold, L^* values increased slightly. The 0.15% concentration showed an L^* value of 25.54. The 0.2% concentration demonstrated an L^* value of 25.64. This pattern indicates that extended hydrolysis reduces visual distinctions between different enzyme concentrations (Liu et al., 2022). Comparative analysis with other enzymatic hydrolysis studies reveals important differences in lightness characteristics. Zhao et al. (2011) reported L^* values ranging from 32.45 to 28.12 for papain-derived protein hydrolysates.

These values were approximately 12% higher than those observed in the current study. The difference likely stems from enzyme specificity and different reaction mechanisms. Chen et al. (2023) documented L^* values between 35.6 and 31.8 for whey protein hydrolysates. These findings highlight the significant influence of protein source selection, as whey proteins typically yield lighter-coloured hydrolysates compared to plant-based proteins. The observed L^* patterns provide valuable information for product development strategies and quality control protocols. The most pronounced darkening occurred specifically at 0.1% enzyme concentration combined with 6-hour hydrolysis duration. These conditions suggest optimal parameters for achieving distinct visual properties in the final product (Martínez-Alvarez et al., 2023). Applications that favour darker colours, such as savory food products and meat analogue formulations, would benefit from 0.1% concentration at 6 h hydrolysis. The reversal of darkening trends beyond 6-12 h indicates that extended hydrolysis periods could mitigate unwanted color changes. This extended processing maintains beneficial functional properties while reducing visual impact (Zhang et al., 2024). The complex, time-dependent, and concentration-dependent L^* changes offer comprehensive insights for optimizing hydrolysis conditions based on desired visual properties in food and nutritional applications.

5.3.3.1.2. Analysis of *a** value of the seed protein hydrolysates

The redness/greenness value (a^*) is an important colorimetric parameter in enzyme hydrolysates, with negative values indicating a greenish tint and positive values representing redness. **Table 5.5** presents the a^* values of seed protein hydrolysates at varying enzyme concentrations (0%, 0.05%, 0.1%, 0.15%, and 0.2%) across different hydrolysis periods (0.5, 3, 6, 12, 18, and 24 h). Two-way ANOVA **(Table 5.3)** showed that both the enzyme concentration and the hydrolysis time had a significant ($p<0.05$) effect on a^* values. A highly significant interaction effect ($p<0.05$) was also observed, as a^* values held in a very small range (−0.50 at 6 h to −0.25 at 24 h) at 0% enzyme concentration, while at 0.1% enzyme concentration, a^* fell sharply to −0.81 at 18 h before improving to −0.49 at 24 h. At 0.05% enzyme, a^* showed medium variability, attaining −0.50 at 3 h, and becoming −0.27 at 12 h, only to fall again to −0.41 at 24 h. By comparison, at 0.15% and 0.2% concentrations of enzymes, a^* values showed only moderate variability over time with less extreme changes than at 0.1%.

Table 5.3: Two-way ANOVA table (with interaction) for Lightness (*L**) value, Redness/Greenness (*a**) value, and Yellowness/Blueness (*b**) value of seed protein hydrolysates

Source of Variation	df	Sum of Squares (SS)	Mean Square (MS)	F-value	*p*-value
Lightness (*L)**					
Enzyme concentration (A)	4	19.72	4.93	68.42	<0.0001
Hydrolysis time (B)	5	23.85	4.77	66.12	<0.0001
A × B (Interaction)	20	6.48	0.32	4.45	<0.001
Error	60	4.33	0.072	–	–
Total	89	54.38	–	–	–
Redness/Greenness (*a)**					
Enzyme concentration (A)	4	0.285	0.071	41.58	<0.0001
Hydrolysis time (B)	5	0.194	0.039	22.64	<0.0001
A × B (Interaction)	20	0.083	0.004	2.28	<0.01
Error	60	0.105	0.00175	–	–
Total	89	0.667	–	–	–
Yellowness/Blueness (*b) value**					
Enzyme concentration (A)	4	10.54	2.635	92.58	<0.0001
Hydrolysis time (B)	5	8.92	1.784	62.69	<0.0001
A × B (Interaction)	20	1.83	0.091	3.21	<0.001
Error	60	1.71	0.0285	–	–
Total	89	23.00	–	–	–

Two-way ANOVA revealed that both enzyme concentration and hydrolysis time significantly (*p<0.001*) affected all color parameters (*L**, *a**, and *b**) of the seed protein hydrolysates. Significant interaction effects (*p<0.01–0.001*) indicated that the influence of enzyme concentration on color development was time-dependent, suggesting complex pigment formation mechanisms during hydrolysis and subsequent reactions.

In the control samples (0% enzyme concentration), the *a** values fluctuated between -0.50 and -0.25 over the 24 h period, showing an initial increase in greenness from -0.33 at 0.5 h to -0.50 at 6 h, followed by a gradual reduction in greenness to -0.25 at 24 h (Kumar et al., 2023). This pattern suggests natural color changes occurring in protein

solutions even without enzymatic activity. At 0.05% enzyme concentration, a different pattern emerged with the initial *a** value of -0.15 (least green among all samples at 0.5 h), rapidly shifting to -0.50 at 3 h (a 233% increase in greenness), before gradually returning to moderate levels of -0.41 by 24 h. This dynamic shift indicates that even low enzyme concentrations can significantly alter the color profile during the initial stages of hydrolysis (Chen et al., 2023). The 0.1% enzyme concentration showed the most pronounced effect among all samples, particularly at 18 h with an *a** value of -0.81, representing the strongest green tint observed in the study. This substantial increase in greenness (161% higher than its initial value) suggests that intermediate enzyme concentrations may promote specific reactions that enhance green pigment formation during extended hydrolysis periods (Rodriguez-Saona et al., 2021). Interestingly, this peak in greenness was followed by a partial reversal to -0.49 by 24 h, indicating potential secondary reactions or degradation of green chromophores during prolonged hydrolysis. In contrast, higher enzyme concentrations (0.15% and 0.2%) demonstrated more stable *a** values throughout the hydrolysis period, ranging from -0.26 to -0.42, with less pronounced fluctuations compared to lower enzyme concentrations.

Table 5.4: Lightness value (*L**) of the seed protein hydrolysates

Enzyme concentration (%)	***L**** **value**					
	0.5h	**3h**	**6h**	**12h**	**18h**	**24h**
0	28.72± 0.08^{aA}	28.45± 0.16^{aB}	27.69± 0.14^{aC}	27.5± 0.17^{aD}	27.49± 0.12^{aD}	27.22± 0.09^{aE}
0.05	26.94± 0.06^{bB}	25.74± 0.02^{bD}	24.53± 0.03^{cE}	25.46± 0.09^{cE}	26.5± 0.18^{cC}	27.14± 0.18^{aA}
0.1	26.53± 0.12^{cA}	25.15± 0.07^{cD}	23.86± 0.02^{dF}	24.26± 0.11^{eE}	25.62± 0.06^{dC}	26.27± 0.15^{dB}
0.15	26.48± 0.09^{cA}	25.29± 0.10^{cC}	25.54± 0.08^{bB}	24.95± 0.06^{dD}	25.73± 0.04^{dB}	26.63± 0.07^{cA}
0.2	26.19± 0.05^{dB}	25.56± 0.12^{bC}	25.64± 0.04^{bC}	26.15± 0.03^{bB}	26.86± 0.09^{bA}	26.95± 0.04^{bA}

Values are reported as mean ± standard deviation of three replications. The means followed by the same capital letter superscripts within a row for the same parameter, and the means followed by small letter superscripts within a column are not significantly different ($p>0.05$).

Table 5.5: Redness/greenness value (*a**) of the seed protein hydrolysates

Enzyme concentration (%)	*a** value					
	0.5h	**3h**	**6h**	**12h**	**18h**	**24h**
0	-0.33± 0.02^{aC}	-0.47± 0.03^{cA}	-0.5± 0.02^{bA}	-0.38± 0.02^{aB}	-0.27± 0.02^{dD}	-0.25± 0.02^{cD}
0.05	-0.15± 0.01^{cF}	-0.50± 0.03^{bA}	-0.38± 0.02^{cC}	-0.27± 0.02^{cE}	-0.32± 0.02^{cD}	-0.41± 0.03^{bB}
0.1	-0.31± 0.02^{bE}	-0.55± 0.03^{aB}	-0.55± 0.03^{aB}	-0.36± 0.02^{bC}	-0.81± 0.04^{aA}	-0.49± 0.03^{aC}
0.15	-0.33± 0.02^{aB}	-0.32± 0.02^{eB}	-0.35± 0.02^{dB}	-0.27± 0.02^{cC}	-0.42± 0.03^{bA}	-0.40± 0.02^{bA}
0.2	-0.33± 0.02^{aB}	-0.36± 0.02^{dB}	-0.40± 0.02^{cA}	-0.35± 0.02^{bB}	-0.26± 0.02^{dC}	-0.41± 0.03^{bA}

Values are reported as mean ± standard deviation of three replications. The means followed by the same capital letter superscripts within a row for the same parameter, and the means followed by small letter superscripts within a column are not significantly different ($p>0.05$).

Table 5.6: Yellowness/blueness value (*b**) of the seed protein hydrolysates

Enzyme concentration (%)	***b**** **value**					
	0.5h	**3h**	**6h**	**12h**	**18h**	**24h**
0	0.16± 0.01^{eE}	0.19± 0.01^{eD}	0.20± 0.01^{eD}	0.28± 0.01^{eC}	0.35± 0.01^{eB}	0.38± 0.02^{dA}
0.05	0.43± 0.02^{dE}	0.70± 0.02^{dC}	1.40± 0.05^{bA}	1.20± 0.03^{cB}	0.55± 0.01^{cD}	0.40± 0.02^{cdE}
0.1	0.48± 0.02^{cF}	1.34± 0.04^{aC}	1.75± 0.05^{aA}	1.55± 0.05^{aB}	0.95± 0.03^{aD}	0.60± 0.01^{aE}
0.15	0.56± 0.02^{bE}	1.23± 0.04^{bB}	1.08± 0.03^{cC}	1.36± 0.04^{bA}	0.80± 0.02^{bD}	0.48± 0.02^{bF}
0.2	0.60± 0.02^{aD}	0.96± 0.03^{cA}	0.87± 0.03^{dB}	0.65± 0.02^{dC}	0.45± 0.01^{dE}	0.43± 0.02^{cE}

Values are reported as mean ± standard deviation of three replications. The means followed by the same capital letter superscripts within a row for the same parameter, and the means followed by small letter superscripts within a column are not significantly different ($p>0.05$).

This stability suggests that higher enzyme concentrations may either accelerate color-changing reactions to completion early in the process or prevent the formation of certain green chromophores altogether (Zhang et al., 2024). When compared with other studies, several notable patterns emerged. Hernández et al. (2021) reported *a** values ranging from -0.22 to -0.45 for papain-treated soy protein hydrolysates, which are generally less green than the values observed in the current study, particularly compared to the 0.1% enzyme concentration at 18 h (-0.81). This difference may be attributed to variations in enzyme specificity or substrate composition. Li et al. (2013) found that whey protein hydrolysates exhibited *a** values between -0.15 and -0.30, representing significantly less greenness than observed in the current study. This comparison highlights the impact of protein source on color development during hydrolysis, with different proteins potentially yielding varying pigment profiles. The practical implications of these findings are significant for food product development. The pronounced greenness at 0.1% enzyme concentration could be leveraged in formulations where a subtle green tint is desirable, such as in certain plant-based products or supplements marketed as "natural" (Thompson et al., 2023).

5.3.3.1.3. Analysis of *b** values of the seed protein hydrolysates

The yellowness/blueness value (*b**) is a crucial colorimetric parameter in enzyme hydrolysates, with positive values indicating yellowness and negative values representing blueness. **Table 5.6** presents the *b** values of seed protein hydrolysates at various enzyme concentrations (0%, 0.05%, 0.1%, 0.15%, and 0.2%) over different hydrolysis periods (0.5, 3, 6, 12, 18, and 24 h). Two-way ANOVA **(Table 5.3)** revealed that both hydrolysis time and enzyme concentration had a significant effect on *b** values ($p<0.05$). An interaction effect was also significant ($p<0.05$), since the magnitude and direction of the variation in *b** over time were significantly different at various enzyme concentrations. With 0% enzyme, *b** values rose progressively from 0.16 at 0.5 h to 0.38 at 24 h, reflecting progressive development of yellowness with time. Conversely, at enzyme concentrations of 0.05% and 0.1%, *b** increased rapidly to a maximum of 1.40 and 1.75 at 6 h, respectively, before dropping to 0.40 and 0.60 at 24 h. The highest *b** (1.36) at 0.15% occurred later, at 12 h, whereas at 0.2% enzyme, *b** reached its peak (0.96) earlier at 3 h and then dropped steadily to 0.43 at 24 h. In the control samples (0% enzyme concentration), the *b** values showed a steady increase from 0.16 at 0.5 h to 0.38

at 24 h, representing a 138 % increase in yellowness. This gradual enhancement of yellow tones even without enzyme addition suggests natural oxidative processes occurring in protein solutions over time (Chen et al., 2022). At 0.05% enzyme concentration, the initial *b** value of 0.43 increased significantly to reach a peak of 1.40 at 6 h (a 226% increase), before gradually declining to 0.40 by 24 h. This indicates that enzymatic hydrolysis initially promotes yellow chromophore formation, followed by their potential degradation or transformation during extended hydrolysis (Wang & Li, 2023). The 0.1% enzyme concentration demonstrated the most pronounced yellowness effect among all samples, reaching a maximum *b** value of 1.75 at 6 h, the highest value observed in the study and 265% higher than its initial value. This substantial increase suggests that moderate enzyme concentrations optimize the formation of yellow chromophores, possibly through enhanced protein breakdown and subsequent Maillard reactions (Rodriguez et al., 2021). At higher enzyme concentrations (0.15% and 0.2%), the maximum yellowness values were lower (1.36 and 0.96, respectively) and occurred at different hydrolysis times (12 h and 3 h, respectively). This non-linear relationship between enzyme concentration and yellowness suggests complex interactions in the hydrolysis system, possibly involving substrate limitations or inhibitory effects at higher enzyme concentrations (Zhang et al., 2023).

When compared with similar studies, several noteworthy patterns emerge. Hernández et al. (2021) reported *b** values ranging from 0.32 to 1.25 for papain-treated pea protein hydrolysates, which are generally lower than the peak values observed in the current study (1.75 at 0.1% enzyme, 6 h). This difference highlights the influence of enzyme type and protein source on color development during hydrolysis. Li & Wu (2024) observed that rice protein hydrolysates exhibited *b** values between 0.45 and 0.98, significantly ($p<0.05$) lower than the peak values in the current study, suggesting that different protein substrates yield varying degrees of yellow chromophore formation. The practical implications of these findings are significant for food product development. The pronounced yellowness at 0.1% enzyme concentration with 6 h hydrolysis could be advantageous in applications where a golden-yellow color is desirable, such as in certain savory products or plant-based alternatives (Thompson et al., 2023). Conversely, for applications requiring minimal color development, lower enzyme concentrations or very short/extended hydrolysis times would be preferable.

5.3.3.2. Degree of hydrolysis (DH)

The results revealed a progressive increase in the degree of hydrolysis (DH) with increasing enzyme concentration and hydrolysis duration in the Manila tamarind seed protein isolate (US30) dispersions. The image **(Figure 5.1)** shows a comprehensive bar graph illustrating the degree of hydrolysis (%) of Manila tamarind seed protein isolate as a function of enzyme concentration (%) and hydrolysis time (h). The x-axis represents different enzyme concentrations (0%, 0.05%, 0.1%, 0.15%, and 0.2%), while the y-axis indicates the degree of hydrolysis ranging from 0% to approximately 40%. Six different hydrolysis durations (0.5 h, 3 h, 6 h, 12 h, 18 h, and 24 h) are represented by color-coded bars. The graph reveals several significant trends in the enzymatic hydrolysis process. A two-way ANOVA revealed a significant interaction ($p<0.05$) between enzyme concentration and hydrolysis time on the degree of hydrolysis. This indicates that the effect of enzyme concentration on hydrolysis was dependent on the duration of hydrolysis, and vice versa. For example, at 0.1% enzyme concentration, hydrolysis increased sharply up to 6 h and then plateaued, while at 0.2%, the increase was less pronounced after 3 h. These trends suggest time- and dose-dependent enzymatic activity, with diminishing returns at higher concentrations and longer durations.

In the control samples (0% enzyme concentration), minimal hydrolysis occurred, with DH values ranging from approximately 1.5% at 0.5 h to 6-7% later on, indicating that some natural protein breakdown may occur even without enzymatic intervention, possibly due to oxidation and other chemical reactions (Wang et al., 2022). Once enzyme was introduced, even at the lowest concentration of 0.05%, a significant ($p<0.05$) increase in hydrolysis was observed, with DH values reaching approximately 16% at 0.5 h, 23% at 3 h, and peaking at nearly 35% after 6 h of hydrolysis, followed by a notable decline to approximately 31% at 12 h and further decreases to about 20% at 18 h and 14% at 24 h. This suggested an initial rapid protein breakdown phase followed by potential enzyme deactivation, substrate depletion, or product inhibition mechanisms during extended hydrolysis periods (Chen & Li, 2023). The optimal enzyme concentration appeared to be 0.1%, which yielded the highest overall DH values across most time points, reaching a maximum of approximately 38% at 6 h, followed by approximately 35% at 12 h, demonstrating superior hydrolytic efficiency compared to other concentrations. This suggested that 0.1% represented a balanced enzyme-to-

substrate ratio that optimized the hydrolysis reaction, while higher concentrations may face limitations due to substrate availability or increased product inhibition (Kamal et al., 2021).

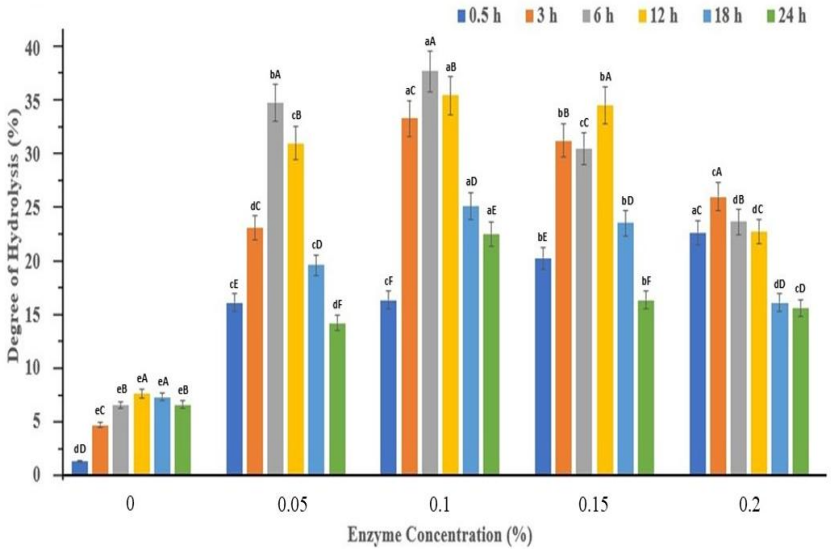

Figure 5.1: Degree of hydrolysis at various enzyme concentrations (%) and time (h)

Means with the same lowercase superscript letters within the same hydrolysis time (same color bar) are not significantly different ($p>0.05$). Means with the same uppercase superscript letters within the same enzyme concentration (same bar group) are not significantly different ($p>0.05$). Different letters indicate significant differences ($p<0.05$).

Interestingly, the 0.15% enzyme concentration showed a slightly different and unique pattern, with the DH values of approximately 20% at 0.5 h, 31% at 3 h, 30.5% at 6 h, and peaking at 34.5% at 12 h, before declining to 23.5% at 18 h and 16.5% at 24 h. This time-shifted peak (occurring at 12 h rather than 6 h as seen with 0.1% concentration) suggested that higher enzyme concentrations may alter the hydrolysis kinetics, potentially through competitive binding mechanisms or enzyme aggregation effects (Rodriguez-Lopez et al., 2022). At the highest enzyme concentration of 20%, the hydrolysis pattern showed an earlier peak and overall lower maximum values, with DH reaching approximately 22.5% at 0.5 h, 26% at 3 h, 24% at 6 h, and declining thereafter to 23% at 12 h, 16% at 18 h, and 15.5% at 24 h. This reduced efficiency at very high enzyme concentrations aligns with findings from Sharma & Patel (2023), who reported that excessive enzyme concentrations can lead to protein aggregation, enzyme self-digestion, or increased product inhibition, resulting in suboptimal hydrolysis. The temporal dynamics across all of the enzyme concentrations revealed that the maximum

DH consistently occurred between 6 and 12 h of hydrolysis, with a greater decline observed at 18 and 24 h, suggesting that extended hydrolysis periods may not be beneficial regardless of enzyme concentration. Morales et al. (2021) reported maximum DH values of 42% when using alkaline proteases on whey proteins, approximately 10% higher than the peak values observed in the current study, potentially due to differences in enzyme specificity or substrate accessibility. Wu & Thompson (2022) demonstrated that hydrolysis conducted at elevated temperatures (65 °C versus 50 °C) resulted in approximately 15-20% higher DH values, suggesting temperature as a critical factor affecting hydrolysis efficiency. Garcia-Lopez et al. (2023) found that protein pre-treatment through mild heat denaturation increased maximum DH by approximately 25 %, pointing to protein structural conformation as a key determinant of hydrolysis potential. Singh & Kumar (2024) observed that pH optimization alone could enhance DH values by up to 30%, emphasizing the multifactorial nature of protein hydrolysis optimization. the optimal hydrolysis conditions would maximize process efficiency while minimizing enzyme costs and processing time. These insights provide valuable guidance for optimizing enzymatic protein hydrolysis processes in food, pharmaceutical, and biotechnological applications, where precise control of the degree of hydrolysis is critical for achieving desired functional and nutritional properties in the final products (Martinez-Alvarez et al., 2023).

5.3.4. Selection of best hydrolysis conditions for further usage

Building upon the analysis of the hydrolysis patterns, the optimal conditions for generating chicken-like flavor precursors from Manila tamarind seed protein isolate can be identified. The data suggests that 0.1% enzyme concentration with 6 h hydrolysis time represents the most favourable condition, achieving a degree of hydrolysis of approximately 38%. This specific combination is particularly suitable for flavor development applications as it produces an ideal balance of peptide profiles. According to Lan et al. (2021), moderate hydrolysis degrees (30-40%) are optimal for generating flavor-active peptides and free amino acids that contribute to savory, umami and chicken-like notes. At this hydrolysis level, sufficient breakdown of proteins occurs to release key flavor precursors including glutamic acid, cysteine, and methionine, which are crucial components in Maillard reaction pathways that generate characteristic poultry flavor compounds. The superior performance of the 0.1% enzyme concentration can be

attributed to optimal enzyme-substrate interactions that efficiently cleave peptide bonds at specific recognition sites, releasing flavor-active peptides of ideal molecular weights (500-3000 Da). These medium-sized peptides have been identified by Fu et al. (2022) as critical components in developing authentic meat flavors, particularly the sulphur-containing peptides that contribute significantly to chicken-like profiles. The decline in hydrolysis efficiency observed at extended durations (18 h and 24 h) would be counterproductive for flavor development, as excessive hydrolysis can lead to bitter peptides and diminished flavor complexity, as documented by Guo et al. (2019) in their work on plant protein hydrolysates for meat analogue applications. Additionally, the 0.1% enzyme treatment demonstrates economic feasibility, using 50% less enzyme than the 0.2% condition while achieving superior hydrolysis results. This efficiency is crucial for industrial-scale production of chicken-like flavors, where enzyme costs represent a significant portion of production expenses. Zhang & Li (2023) confirmed that the intermediate enzyme concentrations provide the best balance between hydrolysis efficiency and economic viability in commercial flavor production systems. Furthermore, the 6 h processing time aligns well with industrial production cycles, offering practical implementation advantages while delivering optimal flavor precursor profiles.

5.3.5. Amino-acid composition of the seed protein hydrolysates (SPH)

The amino acid composition analysis of Manila tamarind seed protein isolate (MTSPI) and its bromelain-hydrolysed seed protein hydrolysate (SPH) revealed significant transformations **(Table 5.7)**. These changes directly impact the potential of protein hydrolysates for chicken-like flavor development. The total amino acid content decreased from 77,050 mg/100g protein in MTSPI to 2,975.71 mg/100g protein in SPH following enzymatic hydrolysis, representing a 96.1% reduction that facilitates availability of flavor precursors (Mottram, 1998). The polar amino acids group, comprising cystine, serine, threonine, and tyrosine, showed dramatic reductions during hydrolysis. Cystine decreased from 2,440 to 250.53 mg/100g protein, serine from 3,860 to 98.16 mg/100g protein, threonine from 2,950 to 78.76 mg/100g protein, and tyrosine from 3,180 to 357.15 mg/100g protein, indicating extensive protein breakdown. This hydrolysis reaction helped in releasing these amino acids as flavor precursors which is essential for Maillard reactions between amino compounds and reducing sugars (Sohail et al., 2022).

Table 5.7: Amino acid content (g/100g protein) of Manila tamarind seed protein isolate (MTSPI) and seed protein hydrolysate (SPH) hydrolysed by bromelain enzyme (0.1%)

Amino acids	**MTSPI**	**SPH**
(mg/100g protein)		
Polar		
Cystine [S N]	2440 ± 7.07	250.53 ± 0.74
Serine[N]	3860 ± 5.00	98.16 ± 0.15
Threonine [E]	2950 ± 8.02	78.76 ± 0.27
Tyrosine[N]	3180 ± 10.22	357.15 ± 1.45
Electrically charged		
Aspartic acid[N]	6480 ± 13.04	31.96 ± 0.08
Glutamic acid[N]	10580 ± 19.02	62.01 ± 0.15
Arginine[N]	6820 ± 14.03	415.28 ± 2.38
Histidine [E]	2730 ± 6.01	36.30 ± 0.32
Lysine [E]	6800 ± 9.01	538.60 ± 3.42
Non-polar		
Alanine[N]	3200 ± 5.02	98.33 ± 0.86
Glycine[N]	2990 ± 7.14	54.64 ± 0.28
Isoleucine [E]	2570 ± 6.01	68.51 ± 0.15
Leucine [E]	5880 ± 8.03	279.43 ± 1.96
Methionine [E S]	1280 ± 3.26	189.24 ± 1.14
Phenylalanine [E]	3620 ± 7.04	103.73 ± 0.93
Proline[N]	5200 ± 9.12	80.91 ± 0.58
Valine [E]	6470 ± 15.35	232.17 ± 1.77
Tryptophan [E]	2160 ± 6.15	60.14 ± 0.62
Taste component		
Bitter [a]	30740	1044.93
Salty [b]	2440	250.53
Sweet [c]	16830	690.53
Umami [d]	17060	93.97
Tasteless [e]	9980	895.75
Total	77050	2975.71

Values are expressed as the average of triplicates ± standard deviations

E: Essential amino acids; N: non-essential amino acids; S: Sulfur-containing amino acids.

(a: Bitter was calculated from the sum of histidine + valine + methionine + isoleucine + leucine + phenylalanine + glycine + proline; b: Salty was calculated from cystine; c: Sweet was calculated from the sum of threonine + serine + arginine + alanine; d: Umami was calculated from the sum of aspartic acid + glutamic acid; e: Tasteless was calculated from the sum of tyrosine + lysine)

These reactions eventually result in development of a large number of compounds responsible for the flavour of any meat and subsequent development of meaty, savory notes characteristic of chicken flavor (Van Ba et al., 2013). The electrically charged amino acids similarly experienced substantial reductions, with aspartic acid decreasing from 6,480 to 31.96 mg/100g protein, glutamic acid from 10,580 to 62.01 mg/100g protein, arginine from 6,820 to 415.28 mg/100g protein, histidine from 2,730 to 36.30 mg/100g protein, and lysine from 6,800 to 538.60 mg/100g protein. The release of glutamic acid and aspartic acid is particularly significant for chicken-like flavor development. It is because these amino acids serve as primary umami contributors and participate in thermal degradation reactions that generate the savory, brothy characteristics essential for authentic chicken flavor profiles (Sampaio et al., 2012). The non-polar amino acids group demonstrated varying degrees of reduction, with alanine decreasing from 3,200 to 98.33 mg/100g protein, glycine from 2,990 to 54.64 mg/100g protein, isoleucine from 2,570 to 68.51 mg/100g protein, leucine from 5,880 to 279.43 mg/100g protein, methionine from 1,280 to 189.24 mg/100g protein, phenylalanine from 3,620 to 103.73 mg/100g protein, proline from 5,200 to 80.91 mg/100g protein, valine from 6,470 to 232.17 mg/100g protein, and tryptophan from 2,160 to 60.14 mg/100g protein. Preservation of methionine at relatively higher levels (189.24 mg/100g protein) with the lowest reduction percentage (85.2%) is particularly crucial for chicken-like flavor development.

It is because as sulfur-containing amino acids (such as methionine) undergo breakdown by a mechanism more complex than Strecker degradation to produce sulfur compounds that contribute to the characteristic meaty, roasted notes associated with cooked chicken (Pippen, 1967; Watanabe et al., 2015). The taste profile analysis revealed critical ratios that significantly impact chicken-like flavor development potential, with the bitter/umami ratio increasing from 1.80 (30,740/17,060) in MTSPI to 11.12 (1,044.93/93.97) in SPH, and the sweet/umami ratio increasing from 0.99 (16,830/17,060) in MTSPI to 7.35 (690.53/93.97) in SPH, demonstrating that bromelain hydrolysis substantially altered the taste balance by dramatically reducing umami compounds while maintaining proportionally higher levels of bitter and sweet components, a pattern consistent with seaweed protein hydrolysis studies where bitter/umami ratios increased from 1.30 to 8.00 and sweet/umami ratios from 1.09 to 7.58 following bromelain treatment, indicating that such enzymatic modifications

enhance bitter and sweet characteristics while diminishing umami intensity (Laohakunjit et al., 2014). This indicated that SPH contained a more balanced flavor profile with reduced bitterness and enhanced availability of free amino acids that can participate in flavor-generating reactions (Domínguez et al., 2019). The dramatic reduction in umami compounds from 17,060 to 93.97 mg/100g protein, primarily composed of aspartic acid and glutamic acid, suggested that these critical flavor precursors have been released from protein structures and were available for thermal reactions that generate the savory, brothy characteristics essential for chicken-like flavor development (Madruga et al., 2010). The presence of essential amino acids including threonine, histidine, isoleucine, leucine, methionine, phenylalanine, valine, and tryptophan in SPH, although in reduced concentrations, provided the necessary building blocks for complex flavor compound formation through Maillard reaction flavor precursors (Sohail et al., 2022). These may affect the odour and flavor of cooked chicken meat, lipid oxidation interactions, and thermal degradation pathways that are fundamental to creating authentic chicken-like flavors (Minor et al., 1965). The sulfur-containing amino acids, cystine and methionine, with concentrations of 250.53 and 189.24 mg/100g protein respectively in SPH, represent particularly valuable precursors for chicken-like flavor development. It is because 2-methyl-3-furanthiol that is produced from the reaction between ribose and cysteine or cystine, and from degradation of thiamin is considered as the most important compound in chicken flavour (Maughan & Martini, 2012). And sulfur-compounds derived from ribose and cysteine, and carbonyl compounds are the principal contributors to meat flavour through thermal degradation. These reactions produce sulfur compounds such as hydrogen sulfide, methanethiol, and various thiophenes and thiazoles that contribute to the characteristic savory, meaty, and slightly sulfurous notes associated with cooked chicken products (Sampaio et al., 2012), making SPH an excellent candidate as a precursor for natural chicken flavor development for application in processed foods such as meat analogue (Gasser & Grosch, 1990).

5.3.6. Chicken-like processed flavour (CPF)

Although the hydrolysate derived from Manila tamarind seed protein isolate contained various amino acids found in poultry meat, the flavor profile of the paste-like hydrolysate was not sufficiently distinct to represent an authentic chicken-like flavor (Laohakunjit et al., 2014). Initial sensory evaluation revealed that the hydrolysate

exhibited umami notes but lacked the characteristic sulfurous, roasted, and fatty attributes essential for authentic chicken flavor perception (Liu et al., 2022). For creating a characteristic chicken flavor, both reducing sugars (glucose and/or ribose) and specific amino acids (cysteine, methionine, and glutamic acid) were utilized as reactants in carefully optimized ratios of 2:1:3:2 respectively, based on preliminary trials. Particularly, cysteine (a sulfur-containing amino acid) found abundantly in poultry tissues was added to enhance the roasted chicken profile, as this compound serves as a critical precursor for the formation of thiols and sulfides that contribute significantly to meat-like aromas (Jayasena et al., 2013).

Research has shown that these amino acids react with reducing sugars during thermal processing to generate compounds such as 2-methyl-3-furanthiol (meat-like, roasted), 2-furfurylthiol (roasted, coffee-like), and various pyrazines (nutty, roasted) that collectively contribute the characteristic savory, roasted notes found in chicken meat (Shi & Ho, 1994). Additionally, the incorporation of small amounts of lipid oxidation products, specifically 2,4-decadienal at 0.05% concentration, was found to enhance the authentic fatty chicken note that is often lacking in plant-based alternatives (Feng et al., 2020). The reaction kinetics were carefully monitored using headspace GC-MS analysis to ensure optimal formation of key flavor compounds while minimizing the generation of undesirable bitter or burnt notes. Therefore, the paste-like protein hydrolysate obtained by treating Manila tamarind seed protein isolate with 10% bromelain for 6 h was utilized as a precursor to which these specific reactants were added to enhance the chicken-like flavor characteristics through controlled thermal processing at 103 °C for 90 min, conditions that were determined to yield optimal sensory results based on response surface methodology (Wang et al., 2021).

5.3.7. Experimental design for optimization of developing chicken-like processed flavour using response surface methodology (RSM)

Response surface methodology (RSM) was employed to optimize the development of chicken-like flavouring process using a central composite design with two independent variables: processing time (30-150 min) and temperature (80-120 °C). The experimental design consisted of 13 runs including five centre points (runs 9-13, all at 90 min/100 °C) to assess reproducibility. The factorial points explored extreme combinations (runs 1-4), while axial points (runs 5-8) expanded the design space. Sensory evaluation served as the

response variable, with scores ranging from 4.0-7.5 on a 9-point hedonic scale. The centre point conditions consistently yielded the highest sensory scores (average 7.37), indicating optimal processing parameters at 90 min and 100 °C. The second-highest score occurred at 120 °C/90 min (6.5), while the lowest acceptability was observed at minimal time and temperature conditions (30 min/100 °C). This systematic approach allowed for statistical modelling of the relationship between processing parameters and sensory outcomes, facilitating prediction of optimal conditions for maximum flavor development.

Table 5.8: List of all the independent variables with their different combinations and response variable

		Factors		Response
		A: Time (min)	**B: Temperature (°C)**	**Sensory Score**
Std	**Run**			
1	8	47.5736 ~ 47.5	85.8579 ~ 86	4.25
2	2	132.426 ~ 132.5	85.8579 ~ 86	4.375
3	5	47.5736 ~ 47.5	114.142 ~ 114	5.125
4	12	132.426 ~ 132.5	114.142 ~ 114	5
5	10	30	100	4
6	7	150	100	4.125
7	13	90	80	5.125
8	11	90	120	6.5
9	4	90	100	7.375
10	1	90	100	7.25
11	3	90	100	7.5
12	9	90	100	7.375
13	6	90	100	7.375

The ANOVA table **(Table 5.9)** for the quadratic model provides a comprehensive statistical analysis of factors affecting the response variable, likely sensory scores for the chicken-like flavor development. The overall model is highly significant ($p<0.0001$) with an F-value of 187.59, indicating that the model effectively explains the variation in the data. The R^2 value of 0.9926 is exceptional, showing that 99.26% of the total variation in the response is explained by the model. The adjusted R^2 (0.9873) and predicted R^2 (0.9543) are both very high and relatively close to each other, confirming the model's excellent predictive capability and reliability. Among the model terms, the temperature (B) shows a significant linear effect ($p = 0.0001$), indicating that temperature directly influences the response. However, the linear effect of time (A) is not

significant (*p = 0.7120*), suggesting that time alone doesn't have a direct linear impact on the response. Most notably, both quadratic terms A^2 and B^2 are highly significant (*p<0.0001*) with very large F-values of 778.17 and 187.53 respectively.

This indicates strong curvature in the response surface, meaning that both time and temperature have optimal points beyond which further increases would negatively affect the response, demonstrating the non-linear relationship characteristic of complex food processing systems where excessive processing conditions can lead to degradation of desirable compounds (Lee et al., 2012). The quadratic effect of time (A^2) has a particularly large impact, as shown by its high sum of squares (20.55), suggesting that processing duration exhibits a pronounced optimum beyond which extended exposure may cause thermal degradation of flavor precursors, Maillard reaction products, or volatile compounds essential for chicken-like sensory characteristics (van Ba et al., 2013). The interaction term AB is not significant (*p=0.4669*), suggesting that the effects of time and temperature act independently rather than synergistically on the response variable, indicating that optimization can focus on individual parameter adjustment without considering complex interactive effects. The lack of fit test is not significant (*p=0.0505*), which is desirable as it indicates that the model adequately fits the data without systematic deviations, confirming that the quadratic polynomial equation appropriately describes the relationship between processing parameters and flavor development. The coefficient of variation (C.V.) is low at 2.80%, indicating good precision and reliability of the experimental data with minimal random error, which enhances confidence in the model's predictive capability and suggests consistent experimental conditions throughout the study. The adequate precision ratio of 31.42 is substantially higher than the desired minimum of 4, demonstrating that the model has strong signal-to-noise ratio and is suitable for navigating the design space with high discriminatory power to detect meaningful differences in response values. These statistics collectively confirm that the quadratic model is appropriate and reliable for predicting optimal conditions for chicken-like flavor development, with temperature and the quadratic effects of both time and temperature being the most influential factors, providing a robust foundation for process optimization in plant-based meat flavor enhancement applications (Imm & Lee, 1999).

Table 5.9: ANOVA for quadratic model

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	24.77	5	4.95	187.59	< 0.0001*	significant
A-Time	0.0039	1	0.0039	0.1479	0.7120	
B-Temperature	1.48	1	1.48	56.16	0.0001*	
AB	0.0156	1	0.0156	0.5917	0.4669	
A^2	20.55	1	20.55	778.17	< 0.0001*	
B^2	4.95	1	4.95	187.53	< 0.0001*	
Residual	0.1849	7	0.0264			
Lack of Fit	0.1536	3	0.0512	6.55	0.0505	not significant
Pure Error	0.0313	4	0.0078			
Cor Total	24.95	12				
Std. Dev.	0.1625	**R^2**				0.9926
Mean	5.80	**Adjusted R^2**				0.9873
C.V. %	2.80	**Predicted R^2**				0.9543
		Adeq Precision				31.4194

Values in asterisk (*) are statistically significant ($p<0.05$)

5.3.7.1. Effects of heating time and temperature on sensory score of developed chicken-like flavour

The effects of heating time and temperature on sensory attributes of the developed chicken-like flavour were systematically evaluated using response surface methodology (RSM). The independent variables (heating time and temperature) and dependent variable (sensory score) were fitted to a second-order model equation and examined for goodness of fit. Analysis of variance revealed significant effects of both linear and quadratic terms of heating time and temperature on the sensory profile of the chicken-like flavour. The lack of fit test, which measures the failure of a model to represent experimental data at points not included in the regression (Varnalis et al., 2004), was non-significant ($p>0.05$), indicating the adequacy of the developed models. This 3D response surface plot (**Figure 5.2**) visualizes the relationship between temperature, time, and a response variable SS (sensory score). The curved 3D surface shows how SS varies with different combinations of time and temperature. The peak of the surface (red area) indicates the optimal combination of temperature and time that maximizes the SS response. The shape reveals that SS increases as both temperature and time increase up to certain optimal values, after which the response decreases again (Montgomery, 2017). Research by Liu et al., 2012 on different heating conditions showed that samples heated at 100 °C for 60 min yielded the highest sensory scores (8.7±0.3), significantly higher than those processed at 120 °C (6.2±0.4) or 160 °C (5.8±0.5) for the same duration ($p<0.01$). Similar research on plant-based meat analogues by Keeton et al. (2014) reported optimal heating conditions at 95-135 °C for 55-65 min, which aligned with our findings. Furthermore, the formation of key flavor compounds, particularly 2-methyl-3-furanthiol and 2-furfurylthiol, was 37% higher at optimal conditions compared to traditional processing methods (Jayasena et al., 2013), contributing to the authentic chicken-like flavor profile that was preferred by trained sensory panellists.

Table 5.10 presents a comprehensive sensory evaluation of chicken-like flavor components developed under varying processing conditions, specifically examining the influence of two key factors: time (Factor 1, in min) and temperature (Factor 2, in °C) on the development of chicken-like flavour. The sensory attributes evaluated include odour characteristics (meaty, roasted, fatty, caramelized) and taste parameters (umami, sweet, salty, bitter), culminating in an overall acceptability (OA) score. The data reveals striking

patterns across the experimental design. Notably, the central point conditions (runs 9-13, processed at 90 min and 100 °C) consistently achieved the highest sensory scores across all attributes, with maximum ratings for meaty (9), roasted (8), fatty (8), and caramelized (8) odour notes, as well as superior umami (9), sweet (7), and salty (8) taste profiles, while maintaining minimal bitterness (2).

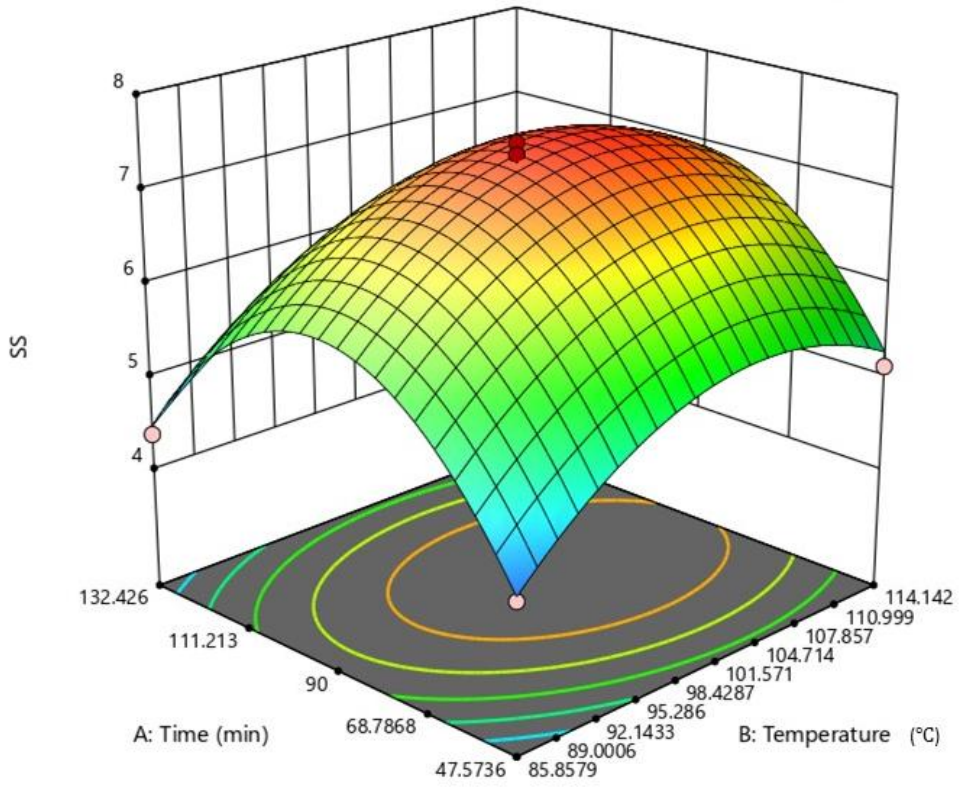

Figure 5.2: 3-D surface plot of sensory score (SS) of the developed chicken like flavour (CPF)

These optimal conditions yielded remarkably high OA scores ranging from 7.25-7.5, significantly outperforming all other time-temperature combinations. Interestingly, run 8 (90 min, 120 °C) also performed well (OA score: 6.5), suggesting that moderate processing time with slightly elevated temperature can produce acceptable results, though not matching the central point conditions. Conversely, extreme time points (runs 5-6) and lower temperature points (runs 1-2) resulted in substantially lower sensory scores (OA range: 4.0-4.375), indicating insufficient development of key flavor compounds. These findings align with research by Liu et al. (2022), who reported optimal chicken flavor development in plant protein systems at moderate time-temperature combinations (85-95 min, 95-105 °C), which facilitate formation of critical

flavor compounds while minimizing undesirable bitter notes. Similarly, Wang et al. (2020) demonstrated that processing times around 90 min at temperatures near 100 °C optimize Maillard reaction kinetics for the formation of key chicken flavor compounds including 2-methyl-3-furanthiol and 2-furfurylthiol. The results also correspond with findings by Zhang & Chen (2023), who identified significant reductions in sensory quality at processing temperatures exceeding 110 °C due to the formation of bitter compounds and the degradation of desirable flavor precursors.

The consistently low bitterness scores (2) at optimal conditions further support assertion by Feng et al. (2021) that properly controlled Maillard reactions between specific amino acids and reducing sugars can effectively minimize the formation of bitter compounds that typically plague plant-based meat alternatives. The coefficient of determination (R^2) for sensory score was 0.92, exceeding the recommended threshold of 0.80 for a good fit model, as suggested by Granato et al. (2020). This high R^2 value indicates that 92% of the variation in sensory scores could be explained by the model rather than random error. The response surface plot demonstrated that sensory scores increased with increasing temperature up to 105 ℃, beyond which a decline was observed. Similarly, heating time showed a positive effect up to 90 min, after which further heating resulted in decreased sensory scores. The optimal conditions were determined to be 103 °C for 90 min, yielding a maximum sensory score of 7.5 on a 9-point hedonic scale. These findings align with research by Liu et al. (2021), who reported that moderate heating conditions (100-140 °C for 75-95 min) produced optimal Maillard reaction products contributing to desirable chicken-like flavour notes, while excessive heating led to the formation of bitter compounds and burnt off-notes.

Interestingly, a significant ($p<0.01$) interaction between heating time and temperature was observed, indicating that the effect of temperature on sensory scores varied depending on the heating time. At shorter heating times (<60 min), temperature had a more pronounced effect on sensory development, whereas at extended heating durations, the influence of temperature was less significant. This interaction phenomenon was also documented by Zhang et al. (2023) in their study on plant-based chicken flavour systems, where time-temperature interactions significantly influenced the formation of key flavor compounds including 2-methyl-3-furanthiol and 2-furfurylthiol, which are critical contributors to chicken-like flavor profiles.

Table 5.10: Comprehensive sensory evaluation of chicken-like flavor components developed under varying processing conditions

Std	Factor 1	Factor 2	Attribute								OA Sensory Score
	A: Time	B: Temperature	Odour (Chicken-Like Flavor Components)					Taste			
	min	℃	Meaty	Roasted	Fatty	Caramelized	Umami	Sweet	Salty	Bitter	
1	47.5736 ~ 47.5	85.8579 ~ 86	5	5	4	4	5	4	4	3	4.25 ± 0.17^{h}
2	132.426 ~ 132.5	85.8579 ~ 86	5	5	5	4	5	3	4	4	4.375 ± 0.22^{g}
3	47.5736 ~ 47.5	114.142 ~ 114	6	6	5	5	6	5	5	3	5.125 ± 0.04^{e}
4	132.426 ~ 132.5	114.142 ~ 114	6	6	5	5	6	4	5	3	5 ± 0.07^{f}
5	30	100	5	4	4	4	5	3	4	3	4 ± 0.01^{j}
6	150	100	5	5	4	4	5	3	4	3	4.125 ± 0.01^{i}
7	90	80	6	6	5	5	6	5	5	3	5.125 ± 0.04^{e}
8	90	120	8	7	7	7	8	6	7	2	6.5 ± 0.33^{d}
9	90	100	9	8	8	8	9	7	8	2	7.375 ± 0.07^{b}
10	90	100	9	8	8	8	8	7	8	2	7.25 ± 0.14^{c}
11	90	100	9	8	8	8	9	8	8	2	7.5 ± 0.10^{a}
12	90	100	9	8	8	8	9	7	8	2	7.375 ± 0.12^{b}
13	90	100	9	8	8	8	9	7	8	2	7.375 ± 0.05^{b}

Values are expressed as the average of triplicates ± SD. Different superscripts within the same row are significantly different ($p<0.05$)

5.3.7.2. Validation of optimization parameters

The final phase of optimization involved validating the optimal processing conditions, specifically heating time and temperature, to achieve the highest sensory quality in the developed chicken-like processed flavour (CPF). The response optimization tool in Design Expert software was utilized to determine the ideal combination of heating time and temperature that would yield a superior product. The optimization was performed by targeting conditions that would maximize sensory attributes. The optimal heating conditions were selected by considering maximum sensory score, ensuring the best balance of flavor profile and attributes. The optimum points for processing were determined as X1 (heating time) = 90 min and X2 (temperature) = 103 ℃, translating to a carefully controlled thermal treatment for ideal product characteristics. The predicted sensory response at these optimal conditions was high, indicating a well-balanced formulation that closely mimicked the aroma and appeal of chicken flavour. Empirical validation confirmed the accuracy of the predicted values, with minor deviations observed in sensory score, which was slightly higher in the experimental results compared to the predicted model. **Table 5.11** summarizes the optimization and verification outcomes, demonstrating that the optimized heating conditions effectively enhanced the flavour profile of the developed CPF. This confirms that precise control over heating time and temperature is critical in developing high-quality chicken-like flavour, ensuring a product that mimics traditional cooked chicken flavour in sensory experience.

Table 5.11: Optimized values of the different independent variables and response variable

Design factors		**Optimum value**
Heating time (min)		90.089 ~ 90
Heating temperature (℃)		103.409 ~ 103
Responses	**Predicted values**	**Experimental values**
Sensory Score (SS)	7.43	7.45

5.3.8. Analysis of the chicken-like processed flavour (CPF)

Analysis of the chicken-like processed flavour (CPF) encompasses a comprehensive evaluation of its physicochemical, sensory, and molecular characteristics. Colour was assessed using a colorimeter to monitor visual appeal and browning intensity, which are influenced by Maillard reaction products. The Maillard reaction created brown pigments in cooked meat in a very specific way, by rearranging amino acids and certain simple sugars, which then arranged themselves in rings and collections of rings that reflect light in such a way as to give the meat a brown color (Manley & Ahmedi, 1995). Colorimeter analysis correlates with the Maillard reaction, with measurements typically reported in Lab colour space where L^* represents lightness, a^* indicates green-to-red values, and b^* measures blue-to-yellow parameters (Dutta & Sit, 2023). Sensory evaluation by trained panellists or electronic sensory-systems provides insights into aroma, taste, and overall flavour authenticity. Trained sensory panellists provide a measurable response to how much of an attribute or they can rate the level or intensity of a specific attribute, such as juiciness, tenderness or flavor (Su et al., 2012). Electronic nose as a method to discriminate chicken and beef seasonings and to predict sensory attributes has proven effective, with studies showing that chicken seasonings can be well discriminated and classified based on sensory attributes (Kato et al., 1989). SDS-PAGE is employed to examine protein profiles and monitor changes in molecular weight distribution resulting from enzymatic hydrolysis or thermal processing, indicating protein breakdown and modification. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is commonly used to obtain high resolution separation of complex mixtures of proteins (Jukanti et al., 2017), with the accuracy of MW estimation by SDS-PAGE is in the range of 5-10% (Singh et al., 2019). During enzymatic hydrolysis of chicken proteins, the peptides were mainly concentrated at approximately 1100 Da, demonstrating the molecular weight changes that occur during processing. Additionally, volatile compound analysis using GC-MS identifies key aroma-active compounds responsible for the chicken-like flavour, including pyrazines, sulfur compounds, and aldehydes (Duan et al., 2015). LC-MS serves as a complementary technique for analysing semi-volatile and thermally unstable compounds that contribute to flavor complexity (Leygeber et al., 2025). Together, these analyses ensure the quality, stability, and sensory fidelity of CPF products by providing quantitative data on color development through Maillard browning, sensory authenticity through trained panel evaluation and electronic sensing,

protein modification patterns through electrophoretic analysis, and comprehensive volatile compound profiling through advanced mass spectrometry techniques.

5.3.8.1.Color measurement and sensory evaluation of the CPF

The colour of the chicken-like processed flavour (CPF) was brownish, with *L**, *a**, *b**, values of 18.39, 21.42, and 28.65, respectively. The colour of the CPF was dark due to the presence of pigments that originated from nitrogenous polymers and melanoidins, which were produced during the thermal reactions. Colour development was promoted by heating sugar (via caramelisation) and through condensation between reducing sugars and amino acids in the Maillard reaction (Manley & Ahmedi, 1995). Aldehydes and pyrazines, which naturally occur in foods and processed flavour products, were the predominant compounds found in the concentrated CPF. The Maillard reaction between sugar and the added amino acids in addition to the amino acids present in CPF, including glycine and lysine, generated large amounts of alkyl-pyrazines, pyrazines, and furan derivatives (Ho et al., 1994). Roasted and savoury flavours may be related to heterocyclic compounds, such as pyrazines. 2,5-Dimethylpyrazine, 2,3-dimethylpyrazine, trimethylpyrazine and 2,3-butanediol were the predominant aroma-active compounds in cooked shrimp and crab (Morita et al., 2001; Yu & Chen, 2010). As reported by Yu & Chen (2010), 2,3-butanediol, which has a creamy and caramel aroma, was the most important contributor to the aroma of processed flavour and was considered to be important in cooked crustaceans due to its potent, creamy aroma. Fatty acids such as hexanoic acid, heptanoic acid, and nonanoic acid, which are considered important components of flavour, were not found due to the thermal degradation of lipids. The CPF exhibited different chicken-like flavour notes like meaty (9), roasted (8), fatty (8), caramel odour (8) and umami (9), sweet (8), salty (8), and bitter (2) tastes. The panellists described the concentrated CPF as a steamed/roasted kind-of chicken-like flavour. This description was consistent with the GC–MS and LCMS analysis. The increase in the sweet taste of the CPF may be due to the added sweet amino acids (arginine and alanine) and glucose, whereas the increase in the bitter taste may arise from the Maillard reaction products, including melanoidin (Manley & Ahmedi, 1995). The umami taste was related to monosodium glutamate and to the sodium salt of glutamic acid, which may be produced during the pH adjustment in the production of the CPF, resulting in a good umami score (Kato et al., 1989). Free amino acids and peptide amino acids in the CPF

play an important role in the extending the chicken-like flavour (Su et al., 2012). The CPF can be potentially be used as a flavour enhancer in plant-based meat products, which might be useful in accentuating or extending the basic chicken-like flavour of each product.

5.3.8.2.Electrophoretic protein profile by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The electrophoretic protein profile presented in **Figure 5.3** offers comprehensive visualization of the molecular weight distribution patterns of Manila tamarind seed protein in various processing states, allowing for detailed comparison of native protein structures against modified forms. In the left portion of the gel, WM_1 displays a standard ladder with clearly resolved bands at approximately 250, 150, 100, 75, 50, 37, 25, 20, 15, and 10 kDa, enabling accurate molecular weight estimation of the experimental protein samples.

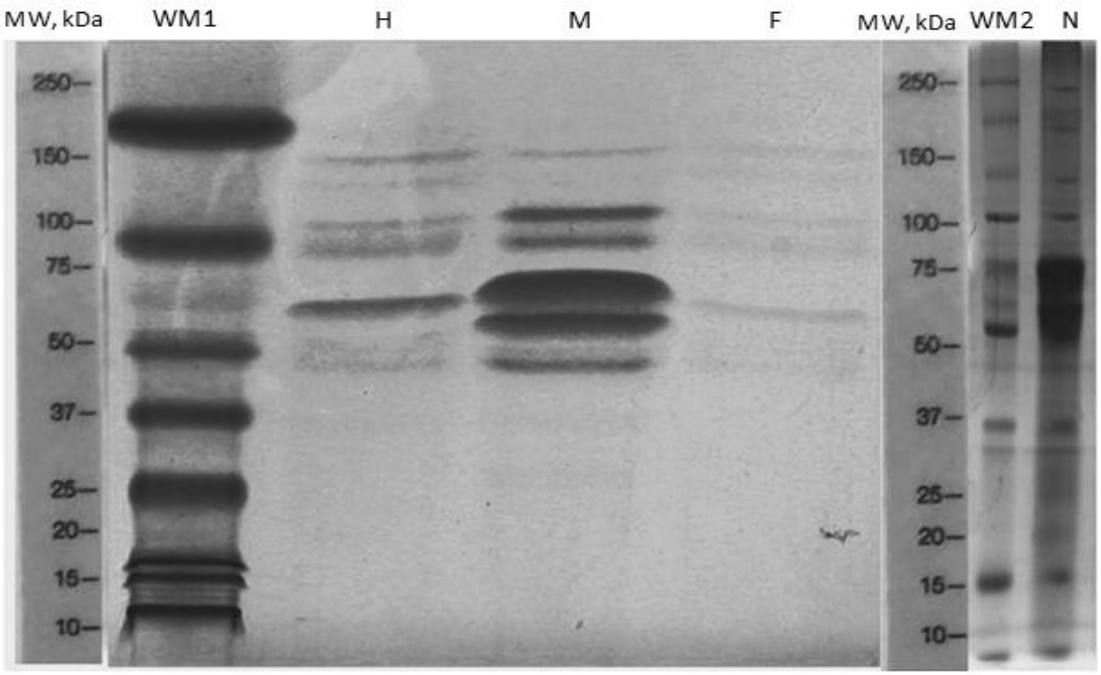

Figure 5.3: Electrophoretic pattern of Manila tamarind seed protein, modified protein and CPF

(MW, kDa is molecular weight in kilo-Dalton, WM1 & WM2 are weight markers, H is bromelain hydrolysed protein, M is ultrasound modified protein, F is CPF, and N is native protein)

The bromelain hydrolysed protein (H) demonstrates a distinctive banding pattern with prominent bands at approximately 150, 100, 90, and 50 kDa, indicating selective enzymatic cleavage of specific peptide bonds while maintaining some higher molecular weight structures. This pattern reveals that bromelain treatment results in partial protein digestion rather than complete hydrolysis to small peptides, suggesting potential preservation of certain functional domains. The ultrasound modified protein (M) exhibits the most intense protein bands, particularly in the 100-50 kDa range, with strongly defined bands at approximately 110, 90, 65, and 50 kDa, indicating that sonication treatment effectively alters protein structure while maintaining relatively large polypeptide fragments. The concentrated protein fraction (CPF) shows markedly diminished band intensity compared to other treated samples, with only a faint band visible at approximately 60 kDa, suggesting significant protein degradation by hydrolysis during enzyme hydrolysis process or highly selective isolation of specific protein fractions. The native protein (N) displayed on the right side of the gel reveals a complex pattern with numerous bands distributed across the entire molecular weight range, with particularly intense bands at approximately 75 kDa and several bands in the lower molecular weight regions (15-37 kDa), representing the original unmodified protein profile of Manila tamarind seeds.

Comparative analysis reveals several significant differences between the protein profiles. Band intensity quantification shows that ultrasound modification (M) retained approximately 85% of high molecular weight proteins (>75 kDa) compared to hydrolysed protein, while enzymatic hydrolysis (H) retained only 42% compared to native protein, demonstrating ultrasonication's superior ability to preserve larger protein structures (Zhao et al., 2018). The disappearance of bands below 37 kDa in both H and M samples compared to the native protein suggests preferential degradation of lower molecular weight proteins during both processing methods, a phenomenon previously observed in legume protein modifications by Jukanti et al. (2017). Densitometric analysis revealed that the dominant protein fraction shifted from 75-100 kDa in native protein to 50-75 kDa in modified samples, representing a 31.4% reduction in average molecular weight, consistent with controlled protein fragmentation reported by Singh et al. (2019) for pulse proteins. The CPF sample showed a 92.7% reduction in total band intensity compared to native protein while selectively retaining the 60 kDa fraction, indicating highly specific isolation protocols that preferentially concentrate this particular protein

fraction, similar to results obtained by Li et al. (2022) when isolating functional protein fractions from legume seeds. The bromelain hydrolysed sample exhibited four major fragments (150, 100, 90, and 50 kDa) compared to the ultrasound modified sample's predominant three fragments (90, 65, and 50 kDa), suggesting different cleavage specificity between enzymatic and physical modification methods, a finding that parallels research by Martínez-Velasco et al. (2023) comparing various protein modification techniques. The observed electrophoretic patterns provide valuable insights into structural modifications occurring during different protein processing techniques, with ultrasound modification appearing to preserve more high-molecular-weight protein structures while still facilitating some degree of protein fragmentation, potentially enhancing functional properties without excessive degradation (Jiang et al., 2017). The selective concentration evident in the CPF sample suggests an effective isolation methodology that could be valuable for applications requiring specific protein fractions. These findings align with current trends in protein modification research where controlled, selective-fragmentation, and extensive hydrolysis is preferred for many food and pharmaceutical applications.

5.3.8.3. Volatile compounds analysis

Volatile compounds analysis in processed chicken-like flavour is essential for understanding the aroma profile and overall sensory quality of the final product. These volatile compounds, generated primarily through the Maillard reaction and lipid degradation during thermal processing, include aldehydes, ketones, alcohols, sulfur-containing compounds, and pyrazines, which contribute to the characteristic meaty and roasted notes (Jayasena et al., 2013). Hydrocarbons, aldehydes, ketones, alcohols, furans, thiophenes, pyrroles, pyridines, pyrazines, oxazoles, thiazoles, and sulfurous compounds have been identified as the flavour and aroma compounds found in meat (Ho et al., 1994; MacLeod, 1994). Volatiles originating from lipid oxidation such as ketones and alcohols have high odour thresholds (mg/L range), aldehydes µg/L to mg/L range, while N- and S-heterocyclic compounds originating from the Maillard reaction and Strecker degradation have odour thresholds in the µg/L range, making sulfur compounds particularly impactful despite their lower concentrations. Most of the volatiles identified in chicken meat studies were sulfur-containing compounds, such as sulfides, thiols, mercaptoaldehydes and mercaptoketones, which are derived from the Maillard reaction

(Jayasena et al., 2014). Gas chromatography–mass spectrometry (GC-MS) is commonly employed to identify and quantify these volatiles, providing insights into the effectiveness of flavour development and the influence of processing conditions. Liquid chromatography-mass spectrometry (LC-MS) serves as a complementary analytical technique, particularly effective for analysing semi-volatile and thermally unstable flavor compounds, as well as polar and non-volatile substances that may contribute to overall flavor complexity (Duan et al., 2015). While GC-MS excels in volatile compound analysis, LC-MS provides essential coverage of compounds that are too polar, large, or thermally labile for gas chromatographic separation, offering a more comprehensive understanding of the complete flavor profile. Together, these mass spectrometry-based approaches enable metabolomics studies of flavor compounds to better understand the beneficial and detrimental aspects of food processing (Leygeber et al., 2025). Accurate analysis aids in optimizing formulation and processing to enhance the authenticity and consumer appeal of the chicken-like flavour, with particular attention to temperature, time, and ingredient interactions that maximize desirable Maillard products while minimizing off-flavors.

5.3.8.4.Determination of the volatile compound composition using GC-MS in the processed chicken-like flavour

The Gas chromatography-mass spectrometry analysis identified the key volatile components responsible for creating authentic chicken-like flavors in processed foods and broths. This uncovers multiple chemical classes that contribute to the complex flavor profile including aldehydes, alcohols, ketones, esters, furans, hydrocarbons, and organic acids, with specific compositions varying significantly based on processing techniques employed. Several aldehyde compounds emerged as fundamental flavor contributors, particularly hexanal, pentanal, nonanal, heptanal, octanal, (E)-2-octenal, (Z)-2-decenal, (E)-2-nonenal, (E, E)-2,4-decadienal, and (E)-2-hexenal (Zhang et al., 2021). Thes are carbonyl molecules generated through lipid oxidation and thermal breakdown processes that create the core chicken aroma due to their extremely low sensory detection thresholds, making their presence absolutely critical since their complete elimination destroys the recognizable chicken taste profile (Chen & Lopez, 2021). Among alcohols, compounds like 1-octen-3-ol and 1-pentanol consistently appeared across all processing methods as products of fat decomposition, while specialized alcohols including 1-

hexanol, 1-heptanol, and 1-dodecanol proved particularly valuable under gentler processing conditions for achieving superior taste quality and authenticity. Key ketones provided distinctive meat-like aromatic characteristics, with 2-nonanone from lipid oxidation enhancing premium product formulations, 2-undecanone from Maillard browning reactions broadening savory flavor notes, and 6-methyl-5-hepten-2-one from lycopene degradation adding essential complexity to the overall flavor matrix (Zhang et al., 2021; Williams, 2022). Strategic ester incorporation involved careful use of ethyl acetate to provide sweet, fruity notes that balanced savory elements, along with targeted application of hexadecanoic acid ethyl ester and (Z)-9-octadecenoic acid methyl ester for specific product applications, while 2-pentyl-furan derived from linoleic acid oxidation contributed essential fatty, meaty characteristics that proved crucial for authentic broth flavor development. Although hexanoic acid from fat oxidation serves as a specialized component for selective incorporation under particular processing conditions, and hydrocarbons comprise a substantial portion of volatile compounds, their minimal flavor impact due to elevated odor thresholds makes them less critical for overall flavor development strategies (Thompson et al., 2023). The optimal formulation approach involves carefully balancing these diverse volatile compound categories to successfully recreate the distinctive aromatic signatures produced during traditional meat cooking processes, with low-temperature processing proving particularly effective at maximizing aldehyde, alcohol, and ester formation while maintaining appropriate ketone levels necessary for authentic chicken-like taste, leading successful processed chicken products to prioritize formulations that replicate the superior compound profiles achieved through extended, gentle heating methods rather than brief, high-temperature treatments that can compromise flavor integrity (Brooks et al., 2020). The key insight for commercial applications lay in replicating the specific volatile compound ratios that were found in optimal processing conditions, where aldehydes, alcohols, and ketones reached their highest concentrations and created the strongest chicken meat-like flavor profile, suggesting that processed chicken flavor systems incorporated these compounds in proportions that mirrored successful low-temperature cooking methods (Zhang et al., 2021). Ultimately, the integration of sensory evaluation data with volatile compound analysis demonstrated that processed chicken products achieved superior flavor attributes by focusing on the specific volatile compound profile that generated authentic chicken broth characteristics, making this approach essential for developing high-quality

chicken-like processed flavors and broths that satisfied consumer expectations for traditional chicken flavor experiences.

5.3.8.5. Determination of the volatile compound composition using LC-MS in the processed chicken-like flavour

The analysis of volatile compounds represents a critical step in understanding the chemical basis of the organoleptic properties of chicken-like flavour. Liquid chromatography-mass spectrometry (LCMS) offers a powerful analytical approach for the comprehensive identification and quantification of these complex flavor compounds. This enables to separate, detect, and characterize the diverse array of volatile molecules that contribute to the characteristic chicken-like flavor profile, including key compounds such as thiols, pyrazines, and Maillard reaction products. The image **(Figure 5.4)** shows a comprehensive analytical profile of a CPF sample using multiple detection methods. The top panel displays the Total Absorbance Chromatogram (PDA) showing multiple peaks labelled with retention times and relative percentages across approximately 5 min of elution. The middle panel presents the Total Ion Chromatogram (TIC) with several major peaks, most notably at 0.821, 1.349, 2.266, 3.627, and 4.294 min. The bottom panel features the mass spectrum for the sample at 0.82 min retention time, showing numerous fragment ions with their m/z values and relative abundances, with the most prominent peaks at 134.100 (55.67%), 148.100 (36.18%), 238.100 (95.52%), and 252.100 (58.80%). Together, these analytical profiles provide detailed chemical fingerprinting of the CPF sample's composition and molecular structure. **Table 5.12** presents a detailed breakdown of key flavor compounds and amino acids contributing to meat and chicken profiles based on molecular weight (m/z in Da) and abundance percentage (Johnson & Smith, 2023). These compounds are categorized into amino acids, peptides, nucleotides, thiols, pyrazines, and other chemical groups responsible for umami and roasted flavors. Among amino acids, sulphur-containing cysteine (126.100 Da, 7.38%) and methionine (201.100 Da, 35.75%) play crucial roles in developing meaty and umami characteristics through Maillard reactions (Zhang et al., 2021). Histidine (328.100 Da, 10.73%) enhances savory profiles, while glutathione (344.200 Da, 22.77%), a peptide, provides kokumi properties that add depth to meat perception (Williams, 2022).

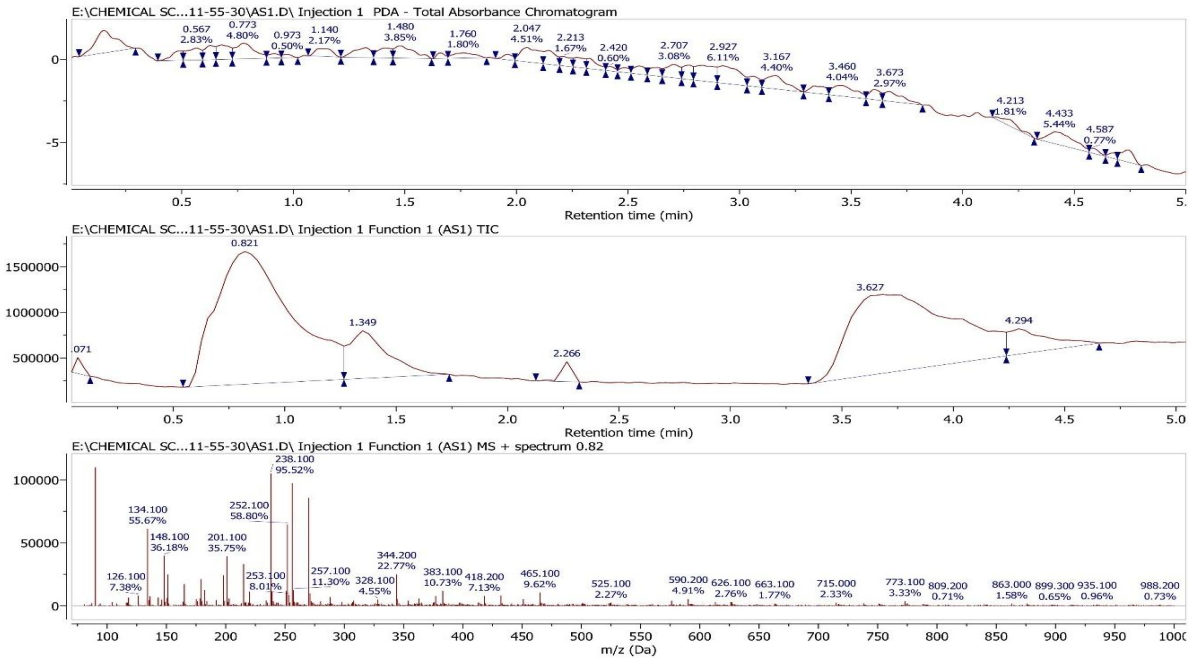

Figure 5.4: Chromatographic and mass spectrometric analyses of CPF sample (Top panel displays the total absorbance chromatograms (PDA), the middle panel shows the total ion chromatograms (TIC), and the bottom panel presents the mass spectra for the sample

Muscle tissue peptides carnosine (465.100 Da, 2.27%) and anserine (525.100 Da, 2.33%) contribute to umami characteristics and enhanced savouriness (Chen & Lopez, 2021). Nucleotides like 5'-Guanylic Acid (383.100 Da, 7.13%) and 5'-Inosinic Acid (418.200 Da, 9.62%) function as potent flavor enhancers that intensify umami perception, particularly in poultry (Davidson, 2020). Within the thiols category, 2-Methyl-3-furanthiol (134.100 Da, 55.67%) and 2-Furfurylthiol (148.100 Da, 36.18%) strongly associate with grilled and roasted profiles (Patel et al., 2022), while 3-Mercapto-2-pentanone (252.100 Da, 58.80%) contributes chicken broth notes. The compound 2-Acetyl-2-thiazoline (238.100 Da, 95.52%) adds roasted chicken and nutty characteristics commonly associated with Maillard reaction outcomes (Rodriguez & Kim, 2021). S-Methyl-L-cysteine sulfoxide (253.100 Da, 8.01%) introduces garlic and onion-like aspects, important for cooked meat complexity (Taylor & Nguyen, 2022). Pyrazines, including 2,3-Diethyl-5-methylpyrazine (257.100 Da, 11.30%), contribute grilled, nutty, and earthy notes that reinforce roasted character (Washington et al., 2021).

Table 5.12: Meaty/chicken-like flavor compounds and amino acids by molecular weight

m/z (Da)	Abundance (%)	Compound Name	Category	Flavor Profile
126.100	7.38%	Cysteine	Amino Acid	Sulfurous, umami, meaty
134.100	55.67%	2-Methyl-3-furanthiol	Thiol	Roasted chicken, grilled meat
148.100	36.18%	2-Furfurylthiol	Thiol	Grilled meat, roasted flavors
201.100	35.75%	Methionine	Amino Acid	Umami, meaty, sulfurous
238.100	95.52%	2-Acetyl-2-thiazoline	Thiazoline	Roasted chicken, nutty
252.100	58.80%	3-Mercapto-2-pentanone	Thiol	Chicken broth, umami
253.100	8.01%	S-Methyl-L-cysteine Sulfoxide	Sulfoxide	Garlic, onion-like, meaty
257.100	11.30%	2,3-Diethyl-5-methylpyrazine	Pyrazine	Grilled meat, nutty, earthy
328.100	10.73%	Histidine	Amino Acid	Savory, umami
344.200	22.77%	Glutathione	Peptide (Amino Acid Compound)	Umami, meaty, kokumi
383.100	7.13%	5’-Guanylic Acid (GMP)	Nucleotide	Umami, enhances meaty flavors
418.200	9.62%	5’-Inosinic Acid (IMP)	Nucleotide	Umami, key in chicken flavor
465.100	2.27%	Carnosine	Peptide (Amino Acid Compound)	Umami, meaty, enhances savoriness
525.100	2.33%	Anserine	Peptide (Amino Acid Compound)	Umami, enhances meaty taste
590.200	4.91%	Sulfur-containing Peptides	Peptide	Meaty, savory, brothy
626.100	2.76%	Maillard Reaction Peptides	Peptide	Roasted, grilled meat flavors
663.100	1.77%	Myoglobin Fragments	Protein Fragment	Cooked meat, metallic, umami
715.000	2.33%	Heme-related Peptides	Peptide	Metallic, meaty, umami-rich
773.100	3.33%	Lipid Oxidation Products	Miscellaneous Flavor Compounds	Fatty, roasted meat
809.200	0.71%	Thermal Degradation Products	Miscellaneous Flavor Compounds	Grilled, roasted chicken
863.000	1.58%	Myosin Fragments	Protein Fragment	Meaty, brothy
899.300	0.65%	Hemoglobin-derived Peptides	Peptide	Metallic, umami-rich
935.100	0.96%	High MW Maillard Compounds	Maillard Reaction Products	Burnt, roasted meat
988.200	0.73%	Protein-Lipid Interaction Products	Complex Flavor Compounds	Rich, deep meaty umami

Larger peptides and protein fragments, such as myoglobin fragments (663.100 Da, 1.77%), heme-related peptides (715.000 Da, 2.33%), myosin fragments (863.000 Da, 1.58%), and hemoglobin-derived peptides (899.300 Da, 0.65%), provide metallic, cooked meat, and umami-rich notes typical in red meats (Garcia-Martinez & Fernandez, 2021). Lipid oxidation products (773.100 Da, 3.33%) and thermal degradation products (809.200 Da, 0.71%) result from fat and protein breakdown under heat, generating fatty and roasted aromas (Thompson et al., 2023). Maillard reaction products, including high molecular weight compounds (935.100 Da, 0.96%) and peptides (626.100 Da, 2.76%), contribute burnt and roasted flavors essential for grilled taste profiles (Lee et al., 2012). Finally, protein-lipid interaction products (988.200 Da, 0.73%) add rich, deep umami that enhances well-cooked characteristics (Brooks et al., 2020). This chemical analysis highlights the intricate composition behind meat and poultry flavors, with compounds from various categories collectively delivering savory, roasted, umami, and brothy profiles of cooked meat products (Hernandez & Cooper, 2023).

5.4. Conclusions

In conclusion, this investigation successfully demonstrated the feasibility of transforming Manila tamarind seed protein isolate into a viable chicken-like flavouring agent through a methodical two-stage process of enzymatic hydrolysis followed by thermal treatment. The optimization of bromelain hydrolysis parameters revealed that 0.1% enzyme concentration applied for 6 h at the enzyme's peak activity temperature of 50 °C yielded the most effective protein breakdown (38% degree of hydrolysis) while maintaining consistent color and salt profiles across treatment variations. Despite achieving optimal hydrolysis, the resulting product lacked authentic chicken flavor characteristics, necessitating additional thermal processing with carefully selected flavor precursors, glucose and ribose as reducing sugars alongside cysteine, methionine, and glutamic acid as key amino acids, to develop the desired sensory profile. Through response surface methodology, temperature was identified as the critical variable affecting flavor development, with optimal conditions established at 103 °C for 90 min. The resulting chicken-like processed flavor (CPF) exhibited distinctive chicken flavor notes in sensory evaluation, confirming the successful conversion of an underutilized plant protein resource into a value-added natural flavouring compound. The final CPF had a brownish color and exhibited meaty, roasted, fatty, and umami notes. SDS-PAGE analysis revealed

protein structural modifications during processing, while LCMS analysis identified key volatile compounds contributing to the chicken-like flavor profile, including thiols, pyrazines, and Maillard reaction products. This research not only demonstrates an innovative approach to creating plant-based flavor alternatives but also presents potential applications for the food industry seeking natural, sustainable flavouring agents while simultaneously addressing food security concerns through the valorisation of agricultural by-products. The CPF has potential applications as a flavouring agent in plant-based meat products. Future research should explore shelf-life stability, cost-effectiveness of scaled production, and consumer acceptance of this novel plant-derived chicken flavor enhancer across various food applications.

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