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Enzymatic Hydrolysis and Retort Processing of Protein Hydrolysate Essence Soup from Buk-Siam Maejo Hybrid Catfish

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ABSTRACT: This work examined two processing steps for a Buk-Siam Maejo hybrid catfish essence soup, enzymatic hydrolysis and retort treatment. Enzyme dose, temperature, and time were evaluated in a Box–Behnken design using Prote AX, an Aspergillus oryzae protease. The selected hydrolysis condition used 0.10% enzyme at 50 °C for 3 h and gave a yield of 72.00%. For retort processing, temperature, time, and F_0 were varied, and 117 °C for 17 min was selected at a reported F_0 of approximately 9 min. Solids rose slightly and pH fell across the thermal treatments, with little variation in viscosity. Thirty untrained panelists gave the soup an overall liking score of 7.77 ± 1.26. The two commercial comparators scored 4.90 ± 2.00 and 6.47 ± 1.70. The processing conditions produced a soup with limited physicochemical changes during retort treatment and favorable liking in this panel. These quality results provide a basis for further development, with thermal-process and shelf-life validation still needed.

1. INTRODUCTION

Fish essence soup requires attention to odor, clarity, and thickness as well as protein content. Fishy odor, turbidity, and high viscosity can make a liquid product less acceptable. We investigated a soup made from Buk-Siam Maejo hybrid catfish, a cross between Mekong giant catfish (*Pangasianodon gigas*) and striped catfish (*Pangasius hypophthalmus*). Growth of this hybrid has been studied in Thai pond and cage culture [1]. Pond water quality and phytoplankton composition in northern Thai aquaculture depend on environmental factors and the fish species stocked [22], and freshwater fish resources also carry recognized conservation and household value in the Mekong region [23]. Using its flesh for an essence soup provides a processing route beyond sale of the flesh itself. Converting agricultural and processing by-products into higher-value products is an established approach in Thai bioresource research, with examples ranging from furniture-industry bamboo waste and cinnamon leaves to low-grade longan [16, 17, 18], as well as oil extraction from this hybrid catfish itself [2].

Approximately half of the hybrid fish is edible flesh. The remaining fraction and processing residues can be directed to other streams, as reported for residue co-digestion and fermenter-effluent biogas systems [19, 20]. Reported composition ranges are 20–40% protein and 5–10% fat [2]. This material offers a substrate for a liquid protein product, potentially alongside familiar products such as chicken essence and bird's nest beverages. For a soup intended for older consumers, the protein content matters [3]. The fish substrate also presents a formulation problem. The finished soup needs acceptable odor and texture as well as a suitable composition.

The experiment addressed this formulation problem through enzymatic hydrolysis followed by retort processing. Enzyme concentration, reaction time, and temperature were compared using degree of hydrolysis as the response. For the soup obtained from that step, we examined how retort conditions changed solids, pH, and viscosity. A sensory comparison with two commercial products assessed liking and whether fishy odor, bitterness, and saltiness were perceived as appropriate.

2. MATERIALS AND METHODS

2.1 Raw material

The raw material was one-year-old Buk-Siam Maejo hybrid catfish supplied by Life Farmland Co., Ltd., Sansai District, Chiang Mai, Thailand. After cleaning and mincing, the flesh was boiled with an equal proportion of water (1:1) for 5 min for defatting. Cheesecloth filtration and mechanical pressing recovered the flesh, which was maintained at 65–70% moisture. Samples were kept at −18 °C until composition analysis and the processing experiments.

2.2 Enzyme

The Prote AX protease preparation was derived from *Aspergillus oryzae* and had endopeptidase and exopeptidase activities. Cleavage of internal and terminal peptide bonds produces shorter polypeptides and free amino acids [4]. The enzyme was active under neutral and acidic conditions, with an activity optimum at pH 5–7 and 35–55 °C. Such hydrolysis can also alter bitterness and flavor. Heating at 90 °C for 10 min was used for enzyme inactivation.

2.3 Proximate composition

Protein, lipid, ash, and moisture contents were measured because fish composition can vary with age, nutrition, and environmental conditions [5]. Protein was determined by Kjeldahl nitrogen analysis using a nitrogen-to-protein conversion factor. Lipid was measured by Soxhlet extraction with an organic solvent, and ash by heating in a muffle furnace to constant weight. Moisture was determined from the loss in weight during oven drying at 135 °C to constant weight.

2.4 Optimization of enzymatic hydrolysis

Fish flesh (50 g) was heated and mixed with 50 mL of distilled water on a hotplate magnetic stirrer at 1,200 rpm for 15 min [6]. Hydrolysis time, enzyme concentration, and temperature were varied according to the Box–Behnken design in Table 1. After each reaction, the enzyme was inactivated at 90 °C for 15 min. The hydrolysate was filtered and centrifuged at 10,000 rpm and 4 °C for 10 min. Experiments were performed in triplicate, and the recovered samples were analyzed for degree of hydrolysis.

Table 1. Variables and coded levels for the Box–Behnken design used to optimize enzymatic hydrolysis.

Variables	Symbols		Coded levels		
	Uncoded	Coded	-1	0	1
Hydrolysis time (hr.)	X_1	x_1	2	3	4
Enzyme concentration (%w/w)	X_2	x_2	0.05	0.1	0.15
Temperature (°C)	X_3	x_3	45	50	55

2.5 Degree of hydrolysis

A 15-mL portion of hydrolysate was mixed with 15 mL of 20% (w/v) trichloroacetic acid (TCA) and left for 10 min. After centrifugation at 9,000 rpm and 4 °C for 15 min, nitrogen in the supernatant was measured by the Kjeldahl method [5]. Degree of hydrolysis (%DH) was calculated from the ratio of TCA-soluble nitrogen to total nitrogen using Eq. 1.

$$DH\% = \frac{\text{10\%TCA soluble N in sample}}{\text{Total N in sample } (raw\ material)}\ x\ 100$$

2.6 Retort processing

The hydrolysate essence soup was filled into hermetically sealed containers and processed in a retort [7]. The design varied temperature (115, 117, and 119 °C), F_0 (7, 9, and 11), and time (17, 24, and 31 min), as listed in Table 2. A sample without

retort treatment served as the control. Solids, pH, and viscosity were measured to compare the effects of the thermal treatments on product quality.

Table 2. Variables and coded levels used in the thermal-processing design.

Variables	Symbols		Coded levels		
	Uncoded	Coded	-1	0	1
Temperature (°C)	X_1	x_1	115	117	119
F_0	X_2	x_2	7	9	11
Time (min)	X_3	x_3	17	24	31

2.7 Total solids

Approximately 5 g of sample was dried at 105 °C to constant weight. Total solids were calculated as the dry weight divided by the initial sample weight and expressed as a percentage [4].

2.8 pH

Samples were measured at 25 °C with a digital pH meter calibrated using standard buffer solutions before analysis.

2.9 Viscosity

Samples were equilibrated to 25 °C and measured with a rotational viscometer. Viscosity was expressed in centipoise (cP).

2.10 Statistical analysis

Means and standard deviations were calculated from triplicate experiments (n = 3). Analysis of variance (ANOVA) was followed by Duncan's multiple range test, with $p < 0.05$ as the significance criterion. For hydrolysis optimization, the Box–Behnken data were analyzed by response surface methodology (RSM). A quadratic polynomial described %DH in relation to enzyme concentration, temperature, and reaction time. ANOVA assessed the model and regression coefficients at the 95% confidence level. R^2 and the lack-of-fit test were used to examine model adequacy.

2.11 Sensory evaluation

The sensory test compared the developed soup with two commercial products using 30 untrained panelists. Each panelist scored appearance, color, odor, taste, texture, and overall liking on a 9-point hedonic scale. The endpoints were 1, dislike extremely, and 9, like extremely. A score of 6 was the minimum acceptable value for each attribute. For fishy odor, bitterness, and saltiness, panelists also selected one of three Just-About-Right (JAR) categories, too weak, just about right, or too strong. Responses were expressed as percentages in each category. Maejo University's Human Research Ethics Committee approved the protocol (MJUIRB ST014/69). Panelists were informed about the procedures and gave written consent before participating.

3. RESULTS AND DISCUSSION

3.1 Proximate composition of the fish substrate

The fresh-flesh analysis gave 38.29% protein, 7.66% fat, 15.10% moisture, and 3.84% ash (Table 3). Following boiling and defatting, the reported protein content was 88.83%, with 1.59% fat and 1.76% ash. The prepared material thus had a greater relative protein fraction and less lipid. This lipid reduction is relevant to hydrolysate flavor because oxidation during storage can produce off-flavors [4].

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Table 3. Composition of fresh and boiled Buk-Siam Maejo hybrid catfish flesh.

Composition	Fresh (%)	Boiled meat (%)
1. Moisture	15.10	≤ 65
2. Protein	38.29	88.83
3. Fat	7.66	1.59
4. Ash	3.84	1.76

Note: Values are means of three independent determinations (n = 3).

The 88.83% protein reported for the defatted flesh is above the cited ranges of 60–85% for fish hydrolysates and 70–85% for Pangasius protein concentrates [8]. Interpreting that comparison requires a common moisture basis. Defatting lowered fat from 7.66% to 1.59%, and ash also fell, from 3.84% to 1.76%. Lipid removal accounts for part of the greater relative protein fraction. Leaching of soluble minerals during boiling could account for part of the ash reduction. Lower lipid content may reduce the risk of oxidative off-flavors [4], but a composition measurement alone cannot determine storage life.

3.2 Hydrolysis conditions and model fit

The hydrolysis comparison selected 0.10% enzyme relative to protein in the flesh, at 50 °C for 3 h (Fig. 1). Under this condition, yield was 72% of the starting raw-material weight, and the maximum reported degree of hydrolysis was 32.6%.

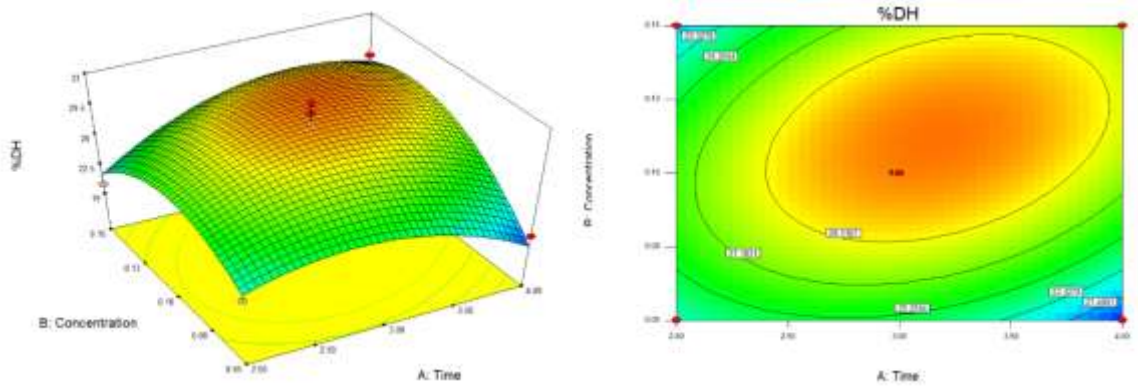


Figure 1. Response surfaces and contours for the effects of hydrolysis time and enzyme concentration on degree of hydrolysis.

Table 4. ANOVA for the hydrolysis model describing degree of hydrolysis.

Source	Sum of squares	*df*	Mean square	*F*-value	Probability (*p*) > *F*
Model	347.57	9	38.62	58.43	<0.0001
X_1-Hydrolysis time	24.52	1	24.52	37.11	0.0015
X_2-Enzyme concentration	89.41	1	89.41	135.34	<0.0001
X_3-Temperature	43.27	1	43.27	65.48	0.0002

X_1X_2	18.21	1	18.21	27.56	0.0034
X_1X_3	0.12	1	0.12	0.18	0.6864
X_2X_3	11.53	1	11.53	17.45	0.0086
X_1^2	76.35	1	76.35	115.58	<0.0001
X_2^2	65.71	1	65.71	99.5	<0.0001
X_3^2	18.45	1	18.45	27.93	0.0032
Pure Error	1.49	3	0.5		
Cor Total	351.54	15			
R^2	0.9887				
R^2 adj.	0.9758				
Lack of Fit	2.48	3	0.83	1.67	0.3245

The quadratic model was significant ($p < 0.05$), with a tabulated R^2 of 0.9887 and adjusted R^2 of 0.9758 (Table 4). The lack-of-fit test was not significant ($p = 0.3245$). Enzyme concentration and temperature had larger effects than reaction time, and the quadratic terms indicated an optimum within the tested range. The enzyme concentration–temperature interaction also mattered, showing why these conditions should be evaluated together. RSM has similarly been used to describe the combined effects of hydrolysis conditions in dogfish muscle [9] and fish soluble concentrate [10]. Statistical optimization of this kind is also common in bioresource processing, where pretreatment and fermentation variables have been tuned to maximize product yield [16, 17, 20]. Hydrolysis with Alcalase has been examined for fish visceral proteins [12], while papain has been studied for mushroom proteins [11]. In the present system, the *Aspergillus oryzae* protease achieved hydrolysis at 50 °C. The experiment did not directly compare its performance with those other enzymes.

Quality changes during retort processing

3.3 Total solids

Total solids increased slightly after retort processing relative to the control (Fig. 2). Changes in broth composition during heating have also been described for clam broth [13] and hydrolyzed fish-based soup [7]. Here, the containers were hermetically sealed, so the increase in solids does not by itself establish moisture loss or a concentration mechanism. The observed difference was small across the tested treatments.

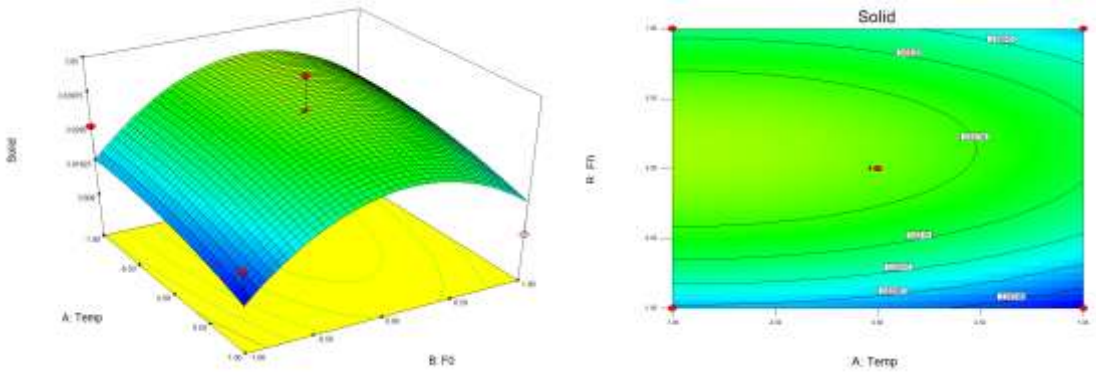


Figure 2. Response surfaces and contours for the effects of F_0 and temperature on total solids.

3.4 pH

Retort-treated samples had lower pH than the control (Fig. 3). Heat-related changes in peptides and other components offer possible explanations. Thermal reactions, including Maillard reactions, can alter the chemical environment of protein

hydrolysates [14], and pH changes have been reported in retort-processed hydrolyzed fish soup [7]. The present pH measurements show a treatment response, but they do not identify the compounds responsible.

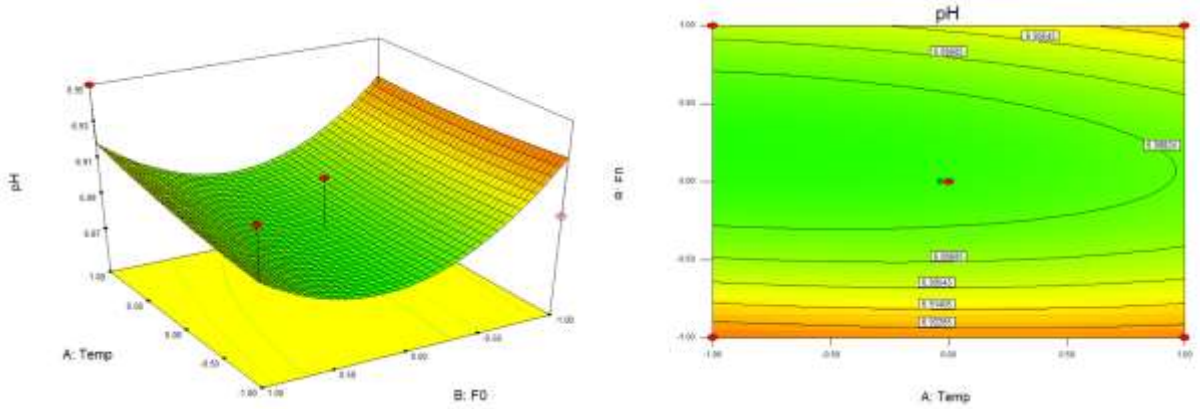


Figure 3. Response surfaces and contours for the effects of F_0 and temperature on pH.

3.5 Viscosity

Viscosity changed little among the retort conditions (Fig. 4). Short peptides produced during hydrolysis may be less susceptible to heat-induced aggregation than intact proteins, although molecular size and aggregation were not measured here. A previous study of hydrolyzed fish soup also reported little effect of retort processing on viscosity and sensory texture [7]. The present results support the consistency of the soup's measured viscosity over this processing range.

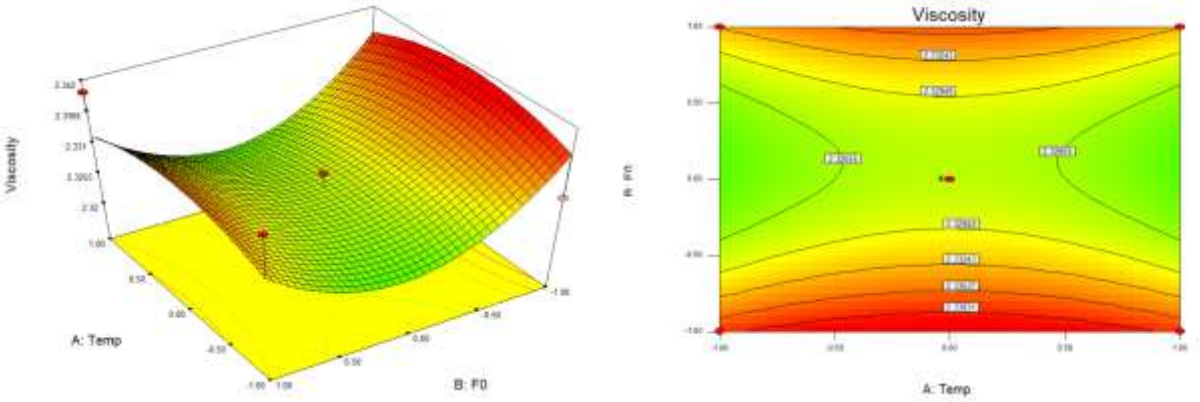


Figure 4. Response surfaces and contours for the effects of F_0 and temperature on viscosity.

3.5 Processing time and product quality

In the retort results, processing time ranged from 9 to 34 min. Total solids increased slightly from approximately 3.61% to 3.66% as processing time increased. Samples processed for 9–12 min had pH values around 6.91–6.94, compared with 6.81–6.85 after 24–34 min. Viscosity decreased only slightly, from 2.34 to 2.30 cP. Thus, the clearest response to longer exposure was the pH decrease, while solids and viscosity varied within narrower ranges. These measurements describe the quality response to processing and do not establish the extent of protein degradation or the sterilization efficacy of a treatment.

3.6 Temperature and F_0

The temperature–F_0 response showed slightly higher solids and lower pH with greater processing severity. Thermal-processing conditions have also been reported to affect the quality of fish products [15]. Higher temperatures combined with shorter exposure have been investigated as a way to limit quality changes [15], but the appropriate combination depends on the product and the delivered heat treatment. For this soup, the selection therefore needs to consider measured product quality together with the thermal process.

3.7 Selection of a retort treatment

A treatment at 117 °C and F_0 = 9 was selected because the soup showed limited changes in solids, pH, and viscosity. Longer thermal exposure produced a larger pH decrease with little additional change in viscosity. These findings provide a product-quality basis for selecting a retort condition. They do not establish energy savings, nutrient preservation, or commercial shelf stability, which were not quantified in this comparison. Where several such criteria must be weighed together, multi-criteria decision frameworks developed for biomass conversion and cascading biorefinery routes offer a structured approach that could be adapted to retort-condition selection [18, 21].

3.8 Sensory results

Thirty untrained panelists compared the soup with the two commercial products. Table 5 gives the hedonic scores for color, aroma, taste, texture, appearance, and overall liking.

Table 5. Hedonic scores for the hydrolysate essence soup and two commercial products.

Attributes	Mean ± SD		
	Protein Hydrolysate Essence Soup	Competitor 1	Competitor 2
Color	7.53 ± 1.15[a]	6.60 ± 1.16[b]	7.47 ± 1.11[a]
Aroma	6.53 ± 1.24[a]	6.47 ± 1.17[a]	6.77 ± 1.04[a]
Taste	6.96 ± 1.47[a]	4.80 ± 1.88[c]	6.83 ± 1.29[a]
Texture	7.21 ± 1.34[a]	5.20 ± 1.63[c]	6.33 ± 1.32[b]
Appearance	7.65 ± 1.14[a]	5.43 ± 1.49[c]	6.23 ± 1.33[b]
Overall	7.77 ± 1.26[a]	4.90 ± 2.00[c]	6.47 ± 1.70[b]

Note: Different superscript letters within a row indicate a significant difference ($p < 0.05$).

Overall liking was highest for the developed soup, at 7.77 ± 1.26, compared with 4.90 ± 2.00 for Competitor 1 and 6.47 ± 1.70 for Competitor 2 (Table 5). Texture and appearance were also higher than both competitors, at 7.21 ± 1.34 and 7.65 ± 1.14. Color (7.53 ± 1.15) and taste (6.96 ± 1.47) were higher than Competitor 1 but shared the same significance grouping as Competitor 2. Aroma scored 6.53 ± 1.24 and did not differ significantly among the products. Some panelists noted a mild fishy odor. In the JAR test, 80% considered the soup's fishy odor just about right and 5% considered it too strong (Table 6).

Table 6. Distribution of JAR responses for fishy odor, bitterness, and saltiness.

Sensory Attributes	Levels	Competitor 1	Competitor 2	Protein Hydrolysate Essence Soup
1. Fishy Odor	Too Weak	5%	10%	15%
	Just About Right (JAR)	40%	70%	80%
	Too Strong	55%	20%	5%
2. Bitterness	Too Weak	2%	15%	20%

	Just About Right (JAR)	30%	65%	75%
	Too Strong	68%	20%	5%
3. Saltiness	Too Weak	25%	15%	5%
	Just About Right (JAR)	65%	75%	85%
	Too Strong	10%	10%	10%

The soup received JAR responses of 75% for bitterness and 85% for saltiness (Table 6). Most panelists therefore considered these intensities appropriate for the tested formulation.

The clearest sensory advantages were in overall liking, texture, and appearance. These differences refer to the 30 untrained panelists and the two commercial products in the test. They provide a reason to continue developing the formulation. Acceptance by the intended older-consumer population requires assessment with that group.

4. CONCLUSION

The selected hydrolysis condition for Buk-Siam Maejo hybrid catfish used Aspergillus oryzae protease at 0.10%, 50 °C, and 3 h. It gave a reported degree of hydrolysis of 32.6% and a yield of 72%. Retort processing slightly increased solids and lowered pH, while viscosity changed little. A treatment at 117 °C and F_0 = 9 was selected for product quality. Overall liking was higher for the soup than for the two commercial comparators, and most panelists judged fishy odor, bitterness, and saltiness to be just about right. Further development needs validated thermal processing and storage performance, together with acceptance testing in the intended older-consumer group.

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AUTHOR CONTRIBUTIONS

Thiphaporn Ruangyot Investigation, Formal analysis, Writing – original draft. **Kanjana Narkprasom** Conceptualization, Methodology, Investigation. **Nukrob Narkprasom** Validation, Data curation, Visualization. **Doungporn Amornlerdpison** Supervision, Resources, Writing – review & editing. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

Ethical approval was not required for this study because it did not involve human participants or animals.

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