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Enzyme Selection for Cobia Fish (*Rachycentron canadum*) Protein Hydrolysate Using a Multiple Criteria Decision-Making Approach

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Abstract

This study applied the Technique for Order Preference by Similarity to an Ideal Solution (TOPSIS), a Multi-Criteria Decision-Making (MCDM) method, to determine the most suitable enzyme for producing protein hydrolysates from Cobia fish *(Rachycentron canadum*). Ten commercial enzymes—ProteAX, M "Amano" SD, A "Amano"2SD, P "Amano" 6SD, Thermoase GL30, SD-NY10, SD-AY10, Papain W-40, Bromelain F, HF "Amano" 150SD—were evaluated at a concentration of 0.2% w w^{-1} under controlled conditions 50°C for 4 hr. The assessment was based on yield, protein concentration, and key sensory attributes, including fishy odor and bitterness, which are critical for consumer acceptance. Given that the hydrolysates were intended for direct consumption in fish broth, sensory characteristics were prioritized. The results identified SD-NY10 as the optimal enzyme, yielding 58 ± 2.0%, with a protein concentration of 3.42 ± 0.27 mg ml^{-1}, and lower intensity scores for fishy odor 3.2 ± 0.24 and bitterness 3.1 ± 0.12, demonstrating the closest CL value 0.970110 alignment with the ideal solution.

Keywords: Fish protein hydrolysate (FPH), Enzyme hydrolysis, Sensory evaluation, TOPSIS

1 Introduction

Cobia (*Rachycentron canadum*), known as Pla Chon Talay in Thailand, is a commercially important marine fish species widely farmed in aquaculture across tropical and subtropical regions worldwide, its rapid growth rate makes it highly economically valuable and high nutrition value (Holt et al., 2007). Recently, Lauteri et al. (2023) reported that ultrasonic processing (60–90 min) improved the tenderization efficiency and firmness of Cobia (*Rachycentron canadum*), enhancing its suitability for high-end culinary applications such as sashimi or restaurant menus. However, sashimi is a delicate dish that must be prepared using extremely fresh seafood. Therefore, a diverse range of innovative processing strategies should be developed to enhance product value and address the varied demands of the market.

Fish protein hydrolysate (FPH) is produced by breaking down fish proteins into smaller peptides and amino acids with varying molecular weights, depending on the extent of hydrolysis using enzymes, acids, or bases (Siddik et al., 2021). FPH has gained significant attention due to its nutritional value and bioactive properties (Chalamaiah et al., 2012). The characteristics and quality of FPH are strongly influenced by factors such as the type of proteases or chemicals used, as well as the temperature, pH, and duration of the hydrolysis process (Nazeer and Kulandai, 2012). Although protein hydrolysates, derived from enzymatic hydrolysis of sources like animal by-products and fish, have numerous applications, they often face the challenge of bitterness (Sujith and Hymavathi, 2011; Fu et al., 2019; Dauksas et al., 2004).

Enzymatic hydrolysis of proteins can improve their functional properties but often results in bitterness, limiting their use in food applications (Daher et al., 2020). This bitterness is primarily associated with hydrophobic amino acid residues in peptides (Idowu and Benjakul, 2019). Fishy flavor often makes products derived from fish less acceptable (Ganeko et al., 2008)

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Amino acids, especially essential ones, influence the fishy odor in fish protein hydrolysates. (Kouakou et al., 2014) Amino acids such as glutamic acid and aspartic acid, prevalent in myosin, contribute to umami flavors, which can mask fishy odors (Hu et al., 2022)

Multi-criteria decision-making (MCDM) is a method that evaluates the alternatives for selection or ranking and choosing the best alternative applicable across various fields like energy and business, it addresses complex decision-making problems across various fields. MCDM has methods that include ELECTRE, PROMETHEE, AHP, SAW, and TOPSIS (Taherdoost and Madanchian, 2023). This study focuses on the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) method, which should have the shortest interval from the positive ideal solution and the farthest distance from the negative ideal solution (Hedayati et al, 2021)

Therefore, this study evaluated ten commercial enzymes from Amano Enzyme Inc. (Aichi, Japan) to identify the most suitable option for producing Cobia fish protein hydrolysate. These enzymes were selected due to their commercial availability and widespread use in the food and biotechnology industries, ensuring feasibility for large-scale production. The assessment was based on key quality parameters, including yield, protein concentration, and sensory attributes such as fishy odor and bitterness. When selecting the optimal food product, various critical factors must be carefully balanced, as some attributes may involve trade-offs.

2 Materials and methods

2.1 Raw material

The fish used in this study was Cobia (Rachycentron canadum), sourced from cage farming in Phuket, Thailand, with an age range of 12–15 months and an average whole fish weight of 6±2 kg. The fillets, weighing 90–100 g per vacuum-sealed bag, were transported in a temperature-controlled vehicle maintained at -18 ± 2 °C to the FACTory Classroom, School of Engineering, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The transportation duration was approximately 15 hr. Upon arrival, the samples were stored at -18 ± 2 °C until further processing to produce protein hydrolysate. The proximate composition of Cobia was analyzed according to AOAC (1993), revealing crude protein content of 19.51%, total fat 10.48%, ash 1.02%, and moisture 68.99%

2.2 Enzymatic hydrolysis

Fish protein hydrolysates (FPH) were produced following a modified method described by Roslan et al. (2014). Ten commercial enzymes, sourced from Amano Enzyme Inc., Aichi, Japan, were utilized in the production process, as detailed in **Table 1**.

Table 1 Type of enzymes.

No.	Enzymes	Preparation derived	Activity
1	ProteAX	Aspergillus oryzae	1,400 U g-1
2	M "Amano" SD	Aspergillus oryzae	40,000 U g-1
3	A "Amano"2SD	Aspergillus oryzae	100,000 U g-1
4	P "Amano" 6SD	Aspergillus melleus	600,000 U g-1
5	Thermoase GL30	Geobacillus stearothermophilus	300,000 U mL-1
6	SD-NY10	Bacillus amyloliquefaciens	70,000 U g-1
7	SD-AY10	Bacillus licheniformis	80,000 U g-1
8	Papain W-40	Papaya	400 U mg-1
9	Bromelain F	Pineapple	800 U mg-1
10	HF "Amano" 150SD	Aspergillus oryzae	150,000 U g-1

stored in sealed packaging at a temperature of 4 °C before use. Cobia fish were minced with 1:1 w v^{-1} water and hydrolyzed at an enzyme concentration of 0.2% w w^{-1}. Then, it was incubated using an incubator shaker (Thermo Scientific, MaxQ 4000, U.S.) at 176 rpm at 50 °C for 4 hr. The enzyme was inactivated using a water bath (WNE Series, Germany) at 90 °C for 25 min, with a magnetic stirrer (2mag magmatic motion MIXcontrol 40, Germany). Centrifuged (UGAIYA, H2050R, Japan) at 10,000 rpm with 5 °C for 10 min to separate the supernatant and subsequently filtered using Whatman filter paper No. 4 (25μm). has been shown in **Figure 1.**

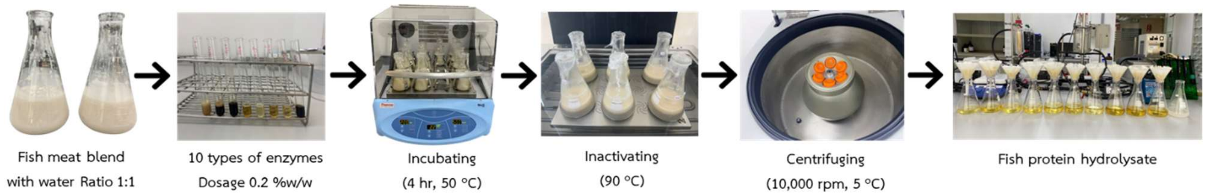

Figure 1 Sample preparation.

2.3 Yield

The yield of protein hydrolysate products was performed according to the method described by Prihanto et al. (2019). Defined as the percentage ratio of the amount of hydrolysate products produced to the number of raw materials used before hydrolysis. The yield is calculated using the following formula Yield (%) = A/B×100

Where A is protein hydrolysate (after centrifugation), and B is the weight of the sample after mixing (before incubation).

2.4 Protein concentration

Determine the protein concentration of supernatant by using Pierce™ BCA Protein Assay Kit (No. 23227), Thermo Fisher Scientific. Calibrator preparation was performed by dilution of 2 mg ml^{-1} bovine serum albumin standard (BSA). The calibrator preparation and the assay processing were performed according to the manual (Thermo Fisher Scientific, 2024)

2.5 Sensory evaluation

Ten trained panelists, comprising staff from the FACTory Classroom Laboratory at KMITL, participated in the sensory analysis. Samples were randomly presented to each

panelist in blind-coded clear glass containers, with each sample consisting of 10 ml. The evaluation was conducted in two sessions.

In the first session, the panelist was asked to rate the samples on scale with sensory attributes including color, mouthfeel, and overall liking of the fish protein hydrolysates (FPH) were assessed using a 9-point Hedonic Scale, where 1 indicated "dislike extremely," 5 indicated "neither like nor dislike," and 9 indicated "like extremely."

In the second session, panelists performed a descriptive analysis to evaluate two specific attributes: fishy odor and bitterness intensity. These were rated on a scale of 0 (no intensity) to 9 (hightest intensity). Panelists were selected based on their sensitivity to fishy odors and flavors, familiarity with fish consumption, and ability to distinguish differences between FPH solutions.

2.6 TOPSIS evaluation method

Firstly, decision-making is arranged in rows and columns. A few variables of sensory (fishy odor and bitterness) are focused on, as the intention is to produce food products, and other qualities of FPH including yield, sensory evaluation (color, mouth feel, overall), and protein concentration are investigated.

The 5 steps of the method are conducted follow by Hedayati et al. (2021); Hedayati et al. (2022)

Step 1 Develop a decision matrix to prioritize and rank the options.

	C_1	…	C_j	…	C_n
A_1	r_{11}	…	r_{1j}	…	r_{1n}
…	…	…	…	…	…
A_j	r_{i1}	…	r_{ij}	…	r_{in}
…	…	…	…	…	…
A_m	r_{m1}	…	r_{mj}	…	r_{mn}

Where Aj illustrates the alternatives j=1,2…m, Cj is the criterion j=1,2…n and rij is the value of each alternative (Aj) and the criterion (Cj)

$$n_{ij}=\frac{r_{ij}}{\sqrt{\sum r_{ij}^2}} \quad (1)$$

Step 2 Calculate the normalized decision-making matrix N. The normalized value n_{ij} is calculated as (1*)*

$$V_{ij}= W_i\times n_{ij} \quad (2)$$

Step 3 Calculate the weighted normalized decision-making matrix where Vij represents the weighted normalized value. Weights are always set subjectively by experts.

$$A^{+} = \{\{\max V_{ij} | j \in B. \{\min V_{ij} | j \in C\}\} \quad (3)$$

$$A^{-} = \{\{\min V_{ij} | j \in B.\{\max V_{ij} | j \in C\}\}$$

Step 4 Determine the negative and positive ideal solution. B is the factor that aims to maximize the objective of the experiment, associated with benefit criteria, and C is the factor that aims to minimize the objective of the experiment, associated with cost.

$$d_i^{+} = \sqrt{\sum_{j=1}^{n} (V_{ij} - V_j^{+})^2} \quad (4)$$

$$d_i^{-} = \sqrt{\sum_{j=1}^{n} (V_{ij} - V_j^{-})^2}$$

Where d_i^{+} and d_i^{-} represent the distance of alternative Ai from the positive and negative ideal solutions, respectively.

$$C_L = \frac{d_i^{-}}{d_i^{-} + d_i^{+}} \quad (5)$$

Where C_L presents relative closeness, Rj ∈ [0, 1].

Step 5 Calculate the relative closeness to the ideal solution and compare R_j values to rank the alternatives. This implies that the value can range from 0 to 1, inclusive. After relative closeness was calculated for each alternative. The higher value of this index means that the alternative is in a better situation.

2.7 Statisticcal analysis

The analysis of variance (ANOVA) was conducted using a significant probability level of 95% ($p < 0.05$) and Duncan's new multiple-range test. The software IBM SPSS Statistics 29 (IBM SPSS, USA) was used to analyze the data.

3 Result and discussion

3.1 Yield and protein concentration of FPH

The present study presents the yield and protein concentration of FPH in **Table 2**. The highest yield and protein concentration were obtained from Thermoase GL30 has high activity, its yield 59±2.5% is lower than Papain W-40 65±1.0% and Bromelain F 62±1.1%, likely due to substrate affinity with enzyme used. According to Noman et al. (2020) The protein hydrolysate yield obtained using papain and Alcalase 2.4 L was reported as 16.77% and 13.30%, respectively. This variation may be influenced by factors such as enzyme specificity, enzyme-substrate affinity, and experimental conditions. Differences in how each enzyme interacts with the substrate can affect the efficiency of hydrolysis, leading to variations in yield. Thus, different enzymes have varying specificities and cleavage patterns, which can impact the amount of peptides and amino acids released during hydrolysis (Abd El-Rady et al., 2023). FPH produced using Alcalase has shown protein concentrations ranging from 89.24% to 92.14%, depending on the specific conditions and raw materials (Dinakarkumar et al., 2022). According to Elavarasan et al (2014) using different proteolytic enzymes, with bromelain-derived hydrolysates showing the most desirable properties of the fish protein hydrolysates were highly dependent on the type of enzyme used, with the hydrolysate prepared using bromelain having the highest functional properties. The type of enzyme used significantly impacts the protein concentration of fish protein hydrolysates.

The conditions under which hydrolysis occur, such as temperature, pH, and time also play a critical role in determining the protein concentration in FPHs

Table 2 Quality properties of different types of enzymes.

Enzyme	Yield (%)	Protein conc. (mg ml^{-1}) *	Color[1]	Mouthfeel[1]	Overall[1]	Fishy odor[2]	Bitterness[2]
ProteAX	60±2.0^{cd}	3.07±0.31^{f}	6.3±0.20^{a}	5.7±0.15^{e}	4.1±0.30^{d}	6.8±0.15^{a}	6.9±0.10^{c}
M “Amano” SD	57±1.0^{f}	2.88±0.20^{g}	6.1±0.15^{c}	5.6±0.24^{e}	4.7±0.24^{b}	3.7±0.20^{g}	5.3±0.25^{g}
A “Amano”2SD	59±1.5^{de}	3.08±0.18^{f}	6.0±0.22^{cd}	5.2±0.15^{g}	3.7±0.20^{f}	5.3±0.26^{e}	6.4±0.15^{e}
P “Amano” 6SD	63±3.0^{b}	3.38±0.25^{d}	5.2±0.23^{f}	5.7±0.32^{e}	3.2±0.21^{g}	6.1±0.15^{d}	6.1±0.35^{f}
Thermoase GL30	59±2.5^{de}	3.60±0.15^{b}	6.2±0.35^{b}	5.7±0.21^{e}	3.0±0.18^{h}	6.5±0.35^{c}	7.3±0.20^{b}
SD-NY10	58±2.0^{e}	3.42±0.27^{c}	6.1±0.10^{bcd}	6.3±0.25^{a}	6.0±0.25^{a}	3.2±0.24^{h}	3.1±0.12^{h}
SD-AY10	57±2.0^{f}	3.09±0.34^{f}	4.4±0.28^{g}	5.8±0.23^{d}	3.8±0.25^{e}	5.3±0.26^{e}	6.7±0.28^{d}
Papain W-40	65±1.0^{a}	3.71±0.30^{a}	5.7±0.36^{e}	6.1±0.22^{c}	3.8±0.10^{e}	6.7±0.14^{b}	7.8±0.32^{a}
Bromelain F	62±1.1^{bc}	3.39±0.25^{cd}	6.0±0.26^{d}	6.2±0.20^{b}	3.9±0.15^{e}	6.8±0.12^{a}	7.8±0.24^{a}
HF “Amano” 150SD	56±1.2^{g}	3.28±0.20^{e}	6.1±0.21^{bcd}	5.4±0.15^{f}	4.4±0.27^{c}	5.1±0.22^{f}	6.9±0.21^{c}

*Protein concentration (mg/ml), [1]Testing by 9-point Hedonic Scale, [2]Testing by descriptive analysis

Data are expressed as mean values ± standard deviation; Values followed by different letters in the same column are significantly different ($P < 0.05$)

3.2 Sensory evaluation

The results are presented in **Table 2.** The sensory attributes are mainly related to the consumer's acceptance. Developing hydrolysates with improved sensory characteristics, such as those enriched with specific amino acids, can further enhance their marketability (Wong et al., 2015). Thus, FPH offers promising benefits, but challenges such as bitterness and rancidity must be addressed to maximize their potential in food products. The results indicated that the SD-NY10 enzyme achieved the highest scores for mouthfeel and overall acceptability, with mean values of 6.3±0.25 and 6.0±0.25, respectively, which were significantly different at ($P<0.05$). On the other hand, the lowest scores were recorded for fishy odor and bitterness intensity, with mean values of 3.2±0.24 and 3.1±0.12, respectively, which were significantly different at ($P<0.05$). Additionally, enzyme activity alone does not solely determine sensory properties. For instance, although Thermoase GL30 exhibits high activity, it resulted in pronounced bitterness 7.3±0.2 and a strong fishy odor 6.5±0.35, leading to lower overall acceptability 3.0±0.18. In contrast, SD-NY10 demonstrated a more balanced hydrolysis pattern, yielding a higher overall acceptability score 6.0±0.25.

Therefore, the SD-NY10 enzyme was the preferred option among customers. According to Steinsholm et al. (2020) different enzymes, such as alcalase and bromelain, affect the sensory profile of FPH. For instance, the study on cod and salmon showed that enzyme choice and hydrolysis time significantly influenced bitterness and other sensory attributes. Gan et al. (2022) investigated the significant impact of enzymatic hydrolysis on the flavor profiles of protein hydrolysates derived from tilapia skin, spine, and head. The study highlighted that those different commercial enzymes, such as Neutrase®, papain, bromelain, and Alcalase®, exhibit varying specificities, influencing the composition of peptides and free amino acids, which ultimately shape key flavor attributes, including umami, bitterness, sourness, and sweetness. Additionally, the degree of hydrolysis (DH) was found to have a proportional relationship with bitterness, emphasizing the importance of processing conditions and raw material composition in determining the sensory properties of protein hydrolysates. Meanwhile, Aspevik et al. (2021) found hydrolysates produced from herring were found to be the most intense flavor, while those made from salmon were considered more palatable. This indicates that the choice of fish species significantly influences sensory characteristics.

3.3 TOPSIS method

Choosing the ideal type of enzyme is challenging because the best properties vary depending on the different criteria being studied. Therefore, the TOPSIS approach was used to handle decision-making with multiple criteria, as it helps identify the best option. The factors including %yield, protein concentration, and sensory properties (color, mouthfeel, overall, fishy odor, and bitterness) were investigated. Firstly, normalized matrices are created using actual values. Next, the matrices are weighted and normalized, with the weights (W) typically set subjectively by experts and the objective of production (Eq. (2). The positive (A+) and negative (A–) ideal solution values of each criterion were determined using a weighted normalized decision matrix (Eq. (3) are shown in **Table 3**

Table 3 Positive (A^+) and negative ideal solution (A^-) for the criteria.

Criteria	W	A^+	A^-
Yield (%)	0.2	0.021812	0.018792
Protein conc.	0.2	0.022553	0.017508
Color	0.05	0.005422	0.005422
Mouthfeel	0.05	0.005459	0.005459
Overall	0.1	0.005422	0.003787
Fishy odor	0.2	0.024527	0.011542
Bitterness	0.2	0.024261	0.009642

Using Eq. 4, the various distances are measured, indicating the position of the different alternatives for each factor from the ideal and negative ideal solutions. Whereas in the context of fishy odor and bitterness, these are attributes where lower scores are preferable. These distances are used in the calculation of relative closeness (Eq. 4). These distances were used for the calculation of relative closeness (CL values), the best alternative 0.970110 had the nearest distance to the positive ideal and the farthest distance to the negative ideal 0.036717 show in **Table 4**. According to the final rankings, enzyme SD-NY10 was the best due to the highest CL value obtained. This result is attributed to sensory properties due to the lowest general score for fishy odors 3.2±0.24 and bitterness 3.1±0.12. The results show that the TOPSIS method can be effectively used in the food industry to simplify comparisons and decision-making.

Table 4 Ranking types of commercial enzymes by TOPSIS.

Enzyme	di^+	di^-	C_L	Rank
ProteAX	0.000348	0.000051	0.127874	7
M “Amano” SD	0.000083	0.000194	0.701305	2
A “Amano”2SD	0.000183	0.000052	0.222084	3
P “Amano” 6SD	0.000204	0.000050	0.197494	5
Thermoase GL30	0.000320	0.000024	0.069874	9
SD-NY10	0.000013	0.000407	0.970110	1
SD-AY10	0.000208	0.000046	0.182624	6
Papain W-40	0.000374	0.000036	0.087384	8
Bromelain F	0.000388	0.000015	0.036717	10
HF “Amano” 150SD	0.000203	0.000055	0.211891	4

4 Conclusion

In conclusion, the TOPSIS analysis, based on criteria including %yield, protein concentration, and sensory attributes (color, mouthfeel, overall acceptance, fishy odor, and bitterness), SD-NY10 was identified as the top-ranked enzyme with the highest consumer acceptance, demonstrating the closest CL value 0.970110 alignment with the ideal solution.

By implementing TOPSIS, the food industry can make more reliable, objective, and strategic enzyme selections, leading to improved efficiency, cost savings, and consumer satisfaction.

These findings suggest that FPH production can be further optimized by refining process parameters. Future studies should focus on optimizing enzyme concentration and incubation time to enhance production efficiency and product quality.

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