

Preparation of a protein drink from fish protein hydrolysate obtained from tilapia skin waste

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Abstract

This study investigated the extraction of protein hydrolysate from tilapia skin waste and its utilisation for the preparation of a protein drink. Tilapia skin was first prepared by removing lipids using 95% ethanol. Protein hydrolysate was then extracted using 0.375% papain at 40°C for 120 mins. The physicochemical properties of the protein hydrolysate were analysed and were then used to prepare a protein drink. The analysis of shelf-life and the prediction of shelf-life of products using the accelerated shelf-life testing method (Q10) were carried out. The content of protein hydrolysate powder in tilapia skin was 29.68±1.32 (g/100 g). Sensory analysis results showed that the protein beverage received an overall liking score of 7.13±1.27 from the sensory panel. Based on the Q10 calculations, the product shelf-life at the recommended temperature of 4°C with a safety factor of 10% was 12 days. According to the Thai FDA for pasteurised beverage regulation no. 195, the product complied with the Thai FDA standard guidelines. These results suggested that the fish protein hydrolysate obtained from tilapia skin waste is a rich resource of protein and is suitable for producing value-added fish skin protein drinks.

1. Introduction

The fish processing industry is a major exporter of seafood and marine products in many countries. Approximately 70% of fish are processed before the final sale (Ghaly *et al.*, 2013). The fisheries sector plays an increasingly important role in the food security and economy of Thailand. Freshwater aquaculture is primarily used for domestic consumption. In Thailand, Nile tilapia (*Oreochromis niloticus*) is one of the main freshwater species produced in large quantities and is economically considered the most important freshwater fish (Islam *et al.*, 2021). In 2016, the aquaculture industry yielded 176,463 tonnes of farm-produced tilapia, of which more than 7,975.4 tonnes of 598.5-million-baht worth were exported (Thai Residents Team, 2017). Of all the exports, 38.1% were in the form of processed frozen and chilled tilapia meat. However, during the production of tilapia, excess waste is generated, which includes skin, scales, fins, and bones, making up over 50%–70% of the raw material (Thai

Residents Team, 2017). The waste volumes depend on the level of processing and type of fish (Ghaly *et al.*, 2013).

Fish are a rich source of nutrients that can impart stability to food products (Wangkheirakpam *et al.*, 2019). There are three types of proteins in fish: structural proteins, sarcoplasmic proteins, and connective tissue proteins. Fish proteins can be extracted via chemical and enzymatic processes. In the chemical method, salts (NaCl and LiCl) and solvents (isopropanol and azeotropic isopropanol) are most commonly used. Enzymatic extraction involves the use of enzymes such as papain, alcalase, neutrase, protex, protemax, and flavorzyme to extract proteins from fish (Ghaly *et al.*, 2013). Fish protein hydrolysates (FPH) are mixed products of polypeptides, dipeptides, and amino acids. They can be produced from protein-containing raw materials by acid reactions, base reactions, or enzymatic hydrolysis (Saputra and Nurhayati, 2016). Protein hydrolysate primarily contains di- and tripeptides, which

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are essential for humans. Proteins are involved in human body function, including skeletal muscle protein anabolism, and are superior to intact (whole) proteins and free amino acids for the skeletal (Manninen, 2009). It has been reported that when the rate of protein synthesis is higher than protein breakdown, the athlete's muscle gain is affected (Kamei *et al.*, 2020). Therefore, it has also been used to develop sports drinks or high-protein beverages for athletes, which are commonly used in this decade (Braspaiboon *et al.*, 2020).

Owing to the growing demand for fish-based tilapia fillet products, large amounts of waste have been generated. The waste is typically discarded, resulting in environmental problems (Arvanitoyannis and Kassaveti, 2008). Recently, Islam *et al.* (2021) reported the data from Phan Fisheries Cooperative, located in Thailand, produced 3.6 tonnes of tilapia waste per year from a total production of 6.0 tonnes per year and discarded waste (gut and intestine) of 1.62 tonnes per year. Conversion of proteins from these wastes to high-value products, such as fish protein hydrolysate, should thus be considered (Srikanya *et al.*, 2017). As mentioned previously, fish protein hydrolysate is a rich resource of protein ($\geq 85\%$) or amino acids that can be used for human consumption (Abraha *et al.*, 2017). New product development from fish waste should be considered as the amount of fish processing by-products discarded each year to reach the appropriate volumes of fish waste utilisation (Islam *et al.*, 2021). This study aimed to utilise protein ingredients obtained from tilapia fish skin waste to prepare a new protein beverage. The findings from this study may help in the development of a new protein drink for athletes from fish skin and thereby increase the number of value products for the beverage industry, reduce fish waste, and improve environmental sustainability.

2. Materials and methods

2.1 Materials

Fresh tilapia skin was purchased from the supermarkets in Chiang Mai, Thailand. Fresh tilapia skin was transported to the laboratory using containers with an ice pack to maintain the temperature at 4°C. Fresh tilapia skin was stored at -18°C to -20°C. The tilapia skin was used immediately within 24 hrs after freezing at -18°C before processing to maintain its quality. Before use, the fresh tilapia skin was thawed (kept in a refrigerator at 4°C for approximately 24 hrs and washed by rinsing with tap water).

2.2.1 Chemical composition analysis

The thawed fresh tilapia skin was treated with 95% ethanol at room temperature (approximately 30°C) for 1 hr to remove lipids. The Association of Official

Agricultural Chemists (2000) methodology was used to determine the moisture, ash, protein, and fat content of fresh tilapia skin. Nitrogen content was quantified using nitrogen combustion. The protein content was calculated by multiplying the nitrogen content by the nitrogen conversion factor for fish skin (6.25).

2.2.2 Enzymatic extraction of fish skin protein

Papain, a widely used enzyme for fish skin protein extraction, was used in this study. Protein hydrolysate was extracted from fish skin following the method of Yarnpakdee *et al.* (2015) and Braspaiboon *et al.* (2020) with some modifications of hydrolysis conditions such as time of hydrolysis. Protein hydrolysate was prepared using 0.375% papain (E.C. 3.4.22.2) (≥ 3 AU/mg) (papaya latex, Sigma-Aldrich, Vienna, Austria) (pH 7.0, 40°C) for 2 hrs. These mixtures were then heated for 15 mins in a water bath at 95°C to inactivate papain. To estimate the moisture content, after drying the protein hydrolysate powder, the samples were placed in an oven at 105°C until a constant weight was obtained. The obtained protein hydrolysate was analysed and used as an ingredient to produce protein drinks.

2.3 Preparation of the protein drink

A new protein drink from tilapia fish skin was prepared, which contained protein hydrolysate obtained from tilapia skin (10 g per serving, 350 mL). The recipe contained vitamins and minerals as per the Thai Food and Drug Administration regulation and was pasteurised at 90°C for 20 s following the Good Manufacturing Practice (GMP) guideline for Electrolyte beverage number 195 (Thai FDA, 2000). The protein drink contained 6–7% glucose and electrolytes (Na^+ , Cl^- , K^+ , and complex B vitamins). The physicochemical characteristics of the protein drinks were analysed following the methods developed by the AOAC (2000). The colour values for all the samples were measured as L-value, a-value, b-value, chroma-value, and hue-value on the Hunter scale using a Minolta colourimeter (Konica Minolta, CR-400 Series).

2.4 Sensory analysis

The 9-hedonic scales of the sensory analyses were used to examine the protein drink (Viriyajaree, 1992). Thirty panellists, who were habitual consumers of protein drinks, were selected as judges for sensory evaluation. The judges were requested to record their degree of preference for the colour, fishy odour, fishy, flavour, sweetness, bitterness, and overall liking according to the 9-point hedonic scale. For the prototype products of protein drinks, sensory evaluation was carried out using a 9-point hedonic scale, together with a 9-point 'just about right' scale. The hedonic scale ranged

from 1 representing ‘dislike extremely’ to 9 representing ‘extremely like’. The ‘just about right’ scale ranged from ‘too weak flavour’ to ‘too strong flavour’. For product development, the 9-point hedonic scale was used with 30 panellists for the same sensory characteristics mentioned above. The products were also evaluated by 30 panellists for product acceptance and the likelihood of purchasing this product in the future. All samples were served in a randomised order in a paper cup and coded with three random digits (Viriyajaree, 1992).

2.5 Shelf-life evaluation

Protein drinks were sampled in triplicate and analysed for seven days at different storage temperatures (25°C, 35°C, and 45°C). The total plate count (TPC) and yeast and mould count were determined during the shelf-life analysis on days 0 and 7, following the method of AOAC (2000). Sensory analysis of the colour and sedimentation of protein drinks during storage was also performed. Q10 was also calculated to evaluate the product shelf-life at the recommended temperature of 4°C. The accelerated shelf-life testing method (ASLT; Q10) was used to measure the quality of the products (Choi *et al.*, 2017) for this study. The Arrhenius equation model was suitable for use because it relates temperature to the reaction velocity (k). To determine the reaction order, a statistical analysis of linear regression was performed (Choi *et al.*, 2017). The Q10-value, which is the reaction rate based on temperature, was defined as the relationship between the reaction rates at (T + 10) and T, as described by Labuza (1982) (see Equations 1 and 2):

$$\ln Q_{10} = (10E_a)/(RT(T+10)) \quad (1)$$

$$Q_{10} = (k_{T+10})/(k_T) \quad (2)$$

where Q10 is the Q10-value, E_a is the activation energy (kcal/mol), R is the gas constant (1.986 cal/mol), k is the rate constant, and T is the absolute reaction temperature (°C).

2.6 Statistical analysis

All measurements were performed at least in triplicates. Means and standard deviations (SD) were calculated. Significant differences were determined by analysis of variance (ANOVA) and Duncan’s multiple range test using SPSS version 15.0. (SPSS Inc., Chicago, IL, USA) (SPSS, 2011).

3. Results

This work investigated the extraction of protein hydrolysate from tilapia skin waste using an enzyme extraction method to produce an ingredient in protein drinks.

3.1 Chemical composition

Tilapia skin was first prepared using a previously described method. As shown in Table 1, tilapia skin contained 70.11±0.85% moisture, 29.68±1.32% protein, 12.07±0.44 % fat, and 0.19±0.08% ash.

3.2 Enzymatic extraction of fish skin protein

The results of the hydrolysis process showed that the specific activity of papain enzyme was approximately 3.28 U/mg. Results from the proximate analysis of protein hydrolysate are shown in Table 1. The protein hydrolysate powder of tilapia skin consisted of 90.97±0.30 g (g/100 g) protein, 2.07±0.44 g (g/100 g) fat, 1.13±0.85% moisture, and 0.37±0.08 % ash.

3.3 Protein drinks

As shown in Table 2, the pH value of the product was 3.41, and the L values corresponded to the lightness of the sample. The L-value of the samples was 75.38 ±0.07. The redness of the samples was measured using the a^* -value. The a^* -value of the sample was 1.32±0.02. The yellowness of the samples was measured using b^* -values. The b^* -values of the sample were 21.05±0.01. The whiteness value of the samples was 67.58.

The prepared protein beverage received an overall liking score of 7.13 ± 1.27 from the sensory panel. The results for overall liking from the panellists (n = 30) revealed that the protein drink had the most appreciated sensorial score for flavour (7.13±1.19) and sweetness (6.83±1.51), followed by colour (6.77± 1.47), bitterness (6.63±1.56), fishy flavour (6.13 ± 1.38), and fishy odour (5.93± 1.61) (Table 3).

FPH in protein drinks was acceptable (Table 4). Based on the comparison of the ‘Like’ to ‘Dislike’ percentage responses from the consumer, the overall acceptability of beverage prepared from FPH on a nine-point hedonic scale indicated that the product was acceptable to 86.67% of the respondents. The FPH of this study received the best acceptance and was liked by the panellists and the overall acceptability was approximately 90%.

The TPC was found to be less than 100 colonies per 1 mL protein drink (Table 5), and healthy protein hydrolysate ingredients were successfully extracted and introduced into beverage industries to boost the country's economy. According to the Thai FDA for pasteurised beverage regulation no. 195, there was no contamination by pathogenic bacteria. However, the number of TPCs should be lower than 100 colonies in a 1 mL sample. There was a non-detectable microbial protein drink or protein drink shelf-life investigation at 25°C, 35°C, and

Table 1. Chemical characteristics of tilapia fish skin and tilapia protein hydrolysate.

Product	Composition value (Mean±S.D.)			
	Moisture (% wb)	Protein* (%)	Fat (%)	Ash (%)
Fish Skin	70.11±0.85	29.68±1.32	2.07±0.44	0.19±0.08
Protein hydrolysate	1.13±0.85	90.97±0.30	2.07±0.44	0.37±0.08

*Conversion factor = 6.25

Table 2. Physico-chemical analysis of the protein drink.

Composition	Protein Drink				
	pH	L*	a*	b*	Whiteness
Value (Mean±S.D.)	3.41	75.38 ±0.07	1.32 ±0.02	21.05 ±0.01	67.58

Table 3. Sensory evaluation of the protein drink

Sensorial Evaluation	Protein Drink						
	Colour	Fishy odour	Fishy flavour	Flavour	Sweetness	Bitterness	Overall
Score (Mean±S.D.)	6.77± 1.47	5.93± 1.61	6.13±1.38	7.13±1.19	6.83±1.51	6.63±1.56	7.13±1.27

Table 4. Acceptance rating of the protein drink.

Description	Panellists	Percentage (%)
Like	26	86.67%
Dislike	4	13.33%

Table 5. Microbiological and sensory analyses of the protein drink stored at various temperatures for seven days

Storage temperature (°C)	Microbiological analysis				Sensory analysis			
	Total plate count (CFU/mL)		Yeast and mould (CFU/mL)		Colour		Sedimentation	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
25°C	N.D.	4	N.D.	N.D.	Normal	Normal	Clear	Little
35°C	N.D.	2	N.D.	N.D.	Normal	Normal	Clear	Little
45°C	N.D.	1	N.D.	N.D.	Normal	Normal	Clear	Little

N.D., means not detectable

45°C for seven days (Table 5). The Q_{10} value calculation results ($Q_{10} = 1.17$) of product shelf-life at the recommended temperature of 4°C with a shelf-life safety factor of 10% was $13.17 \times 0.9 = 11.85 \gg 12$ days.

The results from the sensory analysis showed no change in the colour of protein drink samples during the storage period of seven days at various storage temperatures. No sedimentation was observed in the protein drink samples on day 0, while slight sediment in samples was found on day 7 at all storage temperatures (25, 35, and 45 °C), as shown in Figure 1.

4. Discussion

This study finding was the protein drinks development for tilapia skin waste. The composition of fresh tilapia fish skin was analysed prior to the extraction of the hydrolysate protein using papain. As shown in Table 1, the percentages of moisture, protein, and ash of tilapia skin in this study were similar to the results obtained by Inthuserdha and Chiradetprapai (2015). However, the percentage of fat was higher than the results reported in the research on the extraction of acid-soluble collagen obtained from Nile tilapia (*Oreochromis niloticus*) skin. The results of the chemical properties of

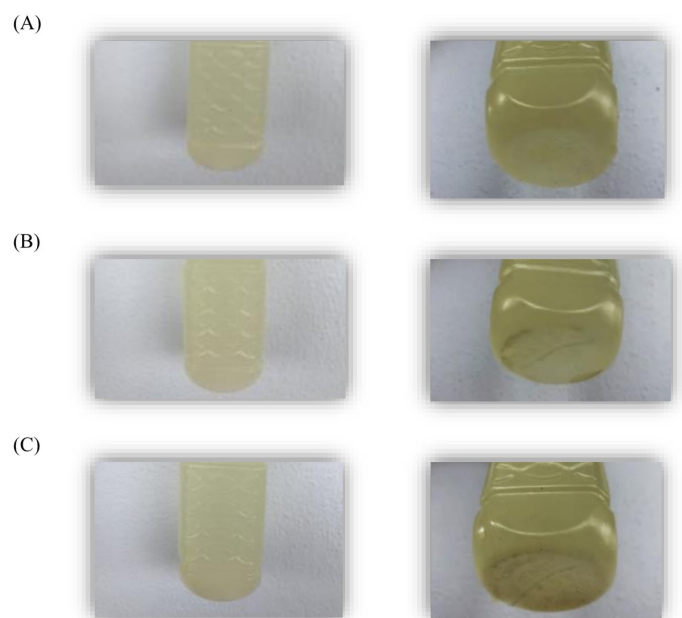


Figure 1. Appearance of the protein drink stored at different temperatures for seven days. Slight sedimentation was observed on day 7, which was attributed to protein precipitation of thermal process at 25°C (A); little sedimentation, 35°C (B); little sedimentation, and 45°C (C); much sedimentation.

fresh tilapia fish skin in terms of moisture, protein, fat, and ash contents were $67.29 \pm 0.77\%$, $30.75 \pm 0.02\%$,

1.64±0.12%, and 0.25±0.03%, respectively. The results of enzymatic extraction of fish skin protein indicated that the product waste of tilapia skin has potential as a major source of FPH. FPH from tilapia skin was obtained by treatment with 0.375% papain (pH 7.0, 40°C) for 2 hrs. The protein content of fish hydrolysate (Table 1) was found to be 90.97±0.30%. Srikanaya *et al.* (2017) converted fish processing wastes from tilapia waste mince (head and frame) into protein hydrolysate using papain enzyme using the optimum conditions of E/S ratio of 1%, 50°C temperature at pH 6.5 for 60 min time of hydrolysis. The protein content was found to be 82.15±0.02%. Thus, it could be concluded that fish protein hydrolysate from this study had high-protein content for application as an ingredient in protein drinks for athletes.

The fish hydrolysate protein extracted from papain was found to be suitable as an ingredient for the production of protein drinks owing to its high nutritional properties. Wisuthiphaet *et al.* (2016) determined the amino acid profile of FPH, that are obtained from the hydrolysis of low-value marine fish using papain, by HPLC. It was found that compared to alcalase, the fish protein hydrolysed by papain had the most suitable nutritional properties; glutamic acid had the highest percentage of 16.35%, followed by 10.41% of aspartic acid and 8.48% of lysine.

The pH value of the protein drinks developed in this study (3.41) complied with the Thai FDA for pasteurised beverage regulation no. 195 as an acidified food product. The L*, a*, and b* values of the product sample were acceptable to the panellists. The results showed that FPH could be used as an ingredient for developing healthy protein beverages, whereas an earlier study reported that fish protein hydrolysate has a problem with bitterness (Abraha *et al.*, 2017). Hydrolysate characteristics may in terms of properties affect the functional properties when used as a food ingredient (Wangkheirakpam *et al.*, 2019). The sedimentation characteristics of product samples result from protein precipitation at low pH, and a small portion of protein can be denatured and aggregated during thermal processing, resulting in a turbid solution (Laclair and Etzel, 2010). The production of beverages along with the presence of hydrolysate collagen (2,000–5,000 Da) helps prevent sedimentation and turbidity (Moskowitz, 2000). According to the Thai FDA for pasteurised beverage regulation no. 195, the number of TPCs should be lower than 100 colonies in a 1 mL sample. The results of microbiological analysis (TPC and yeast and mould count) and shelf-life analysis of protein drinks at 25°C, 35°C, and 45°C for seven days showed that there was no contamination with pathogenic bacteria in the protein drink.

5. Conclusion

FPH is a rich resource of protein that is suitable for producing value-added fish skin protein drinks. The papain enzyme extraction method is an effective method to extract the hydrolysate protein from fish skin. The obtained fish protein hydrolysate can be used as an effective ingredient in protein drinks for athletes with high scores for consumer acceptance. For further study, FPH from fish skin waste could be applied in the beverage industry to increase the use of highly valuable protein hydrolysates from fish waste. Further studies should be conducted to compare the protein drinks from tilapia skin with other commercial protein drinks in athletes.

Conflict of interest

The authors declare no conflict of interest. The funders had no role in the study design; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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