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Study of Protein Concentrate from Flying Fish Roe Filament and its Application for Nutrified Rice-Corn Milk

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Abstract

One of the interesting marine products to be explored is flying fish (*Hirundichthys oxycephalus*) roes. The flying fish roe is usually called *tobiko*. The aim of this study isto extract protein from *tobiko* filaments using an isoelectric point approach, analyze their chemical properties, and apply them to the nutrification of rice-corn milk. Extraction of *tobiko* filaments using an isoelectric point approach resulted in an optimal pH of 8.5 based on the protein content (73.52 ± 0.07 %). Extraction under alkaline conditions (pH 8.5) resulted in a protein concentrate yield of 9.04% and an insoluble portion of 69.79%. That protein concentrate showed 15 amino acid, leucin (5.86 ± 0.01%), lycin (3.69 ± 0.02%), valin (3.41 ± 0.02%), isoleucine (3.33 ± 0.01%), threonine (2.86 ± 0.01%), phenylalanine (2.30 ± 0.02%), histidine (1.38 ± 0.01%), and methionine (1.21 ± 0.01%), glutamate (7.08 ± 0.01%), arginine (6.11 ± 0.01%), alanine (3.82 ± 0.01%), aspartic acid (3.75 ± 0.01%), serine (3.05 ± 0.02%), glycine (1.84 ± 0.01%), and tyrosine (1.46 ± 0.01%). The addition of protein concentrate from *tobiko* filament showed an increase in protein content in rice-corn milk so the purpose of nutrification in this study was successful. The best formulation is in the composition of rice: corn: protein concentrate (15:5:3%) with details of moisture content 65.07 ± 0.02%, ash content 0.50 ± 0.01%, the lipid content 0.28 ± 0.02%, the protein content 21.18 ± 0.02 %, the carbohydrate content 12.95 ± 0.02%, with a total energy 278.13 ± 0.03 kcal.

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Introduction

Exploration of nutritional compounds and bioactive compounds in foodstuffs is always interesting to study. Some research results even show some sources that are not considered, such as waste and by-products of food products, to extract or isolate for something new in nutrition or functional food process. Other than agricultural materials, marine products can be explored for protein and fatty acid sources for several case studies such as stunting,[1] diabetes mellitus,[2] antioxidant,[3] antihypertesive,[4] anticoagulant,[4] immunomodulatory compound,[4] and amongst other functions.

One of the interesting marine products to be explored is flying fish (*Hirundichthys oxycephalus*) roes. The flying fish roe is usually called *tobiko*. *Tobiko* is generally used as a topping in sushi from Asian Cuisine.[5] Indonesia is one of the largest producers of *tobiko*, precisely in South Sulawesi, Makassar Strait. The number of exports of flying fish Roes originating from South Sulawesi reaches 20-30% of the total export of Indonesian fish roes on the Asian continent.[6,7]

Tobiko has a high protein and fatty acid content, so it has been widely researched and developed as a health supplement. The waste from *tobiko* production is the filament that is used as a place for flying fish to lay their roes. *Tobiko* filaments come from white fibers that surround *tobiko*. Four hours after fertilization occurs, flying fish roes will produce fine white filaments to attach between roes so that flying fish roes will clump on marine vegetation. The filament formed resembles fibrous yarn with a low level of elasticity but is very sturdy.[8]

Several studies related to flying fish eggs have been carried out. Flying fish egg filament is known to contain 72-74% protein.[9] Another study reveal resurt that *tobiko* filament have a higher protein than the roe itself, *tobiko* filament contains protein of 40.10% (dry based) and 33.70% (wet based), while *tobiko* contains protein of 37.53% (dry based) and 30.27% (wet based).[10] The high protein content of *tobiko* filament has potential to be developed as a supplement or nutrification material for processed products.

This study performs extract protein from *tobiko* filaments and then make it a nutrification material from non dairy-plant-based-milk.Because it is a preliminary case, the nutrification of non-dairy-plant-based-milk products in this study is focused on increasing the protein content of the product. The nutrified product is expected to be an alternative to milk drinks for people with lactose intolerance. It is known that 75% of the world's population is lactose intolerant.[11,12] This product is expected to be an alternative drink for ages 0-3 years. This age range is commonly used for research on foods for special medical purposes (FSMPs).[13] The basic ingredients chosen are rice and corn. Rice-corn milk has a great potential for future as nutritional food products14 and can be an alternative for non-dairy-plant based15. Rice contains 32-78% protein16 meanwhile corn contains 6-12% protein.[17] Several previous studies have examined the protein profile of plant milk made from rice16 and corn18, so that this research will be interesting if applying protein concentrate from *tobiko* filaments for the purpose of protein nutrification.

The study used a protein extraction method based on the pH approach concerning the isoelectric point of amino acids. The isoelectric point can be used as a basis during the extraction process so that the protein will precipitate when certain pH conditions are following the desired type of amino acid.[19,20] Meanwhile, the *tobiko* filament protein has a high phenylalanine and tyrosine content,[10] both of which are aromatic amino acids.[21] The explanation is based on the research using protein extraction using the technique of opening an alkaline atmosphere. HPLC then tested the extraction results to determine the amino acid profile of the constituents. Proteins from *tobiko* filaments were also applied to rice-corn-based milk for nutrification purposes.

Materials and Method

Materials

This study was used *tobiko* filament from Kelola Mina Laut Madura Indonesia, rice (iherang variety) and sweet corn (bisi sweet 2) from Malang Indonesia. Ingredients for milk are sugar, water, stabilizer CMC (Carboxy Methyl Cellulose). The chemicals used for protein extraction are NaOH (1 M), aquades, and citric acid (1 M). The analysis used BSA reagent (bovine serum albumin), biuret reagent, ethanol (96%), petroleum benzene, alcohol (80%), and HCl (1 M). The tools used include: Spectrophotometer UV-VIS (Shimadzu, 6500), analytical balance

(Sartonius type TE15025), oven (Yamato type DV-41), desiccator, heating (Sibata type SB-6),distillator, burette, furnace (Yamato type FM 38). Tool used for amino acid is HPLC (Thermo Scientific ODS-2 Hyersil).

Research Procedure

Protein Extraction

Protein extraction in pH-based research.[22] In this method, no removal is performed for fat content in the material. The filaments of dried flying fish roes were smoothed or scaled-down. *Tobiko* filament was then added with distilled water (10% w/v) and stirred at room temperature (25˚C) for 5 hours. The sample was then stored in the showcase overnight and filtered to obtain the filtrate. The filtrate obtained was then extracted with the addition of 1 M NaOH until a pH of 8.5 ± 0.2 was obtained and stirred for 4 hours with a magnetic stirrer at room temperature (25˚C). The following is a flow chart of the protein concentrate extraction process. The protein filtrate was then added with 1M citric acid to pH 7 ± 0.2 and centrifuged at room temperature (25˚C) for 20 minutes at 10,000 rpm. The precipitate obtained was then suspended with distilled water to remove the remaining NaOH and citric acid in the precipitate. The suspension was then re-centrifuged (25◦C, 10,000 rpm), and the precipitate obtained was then freeze-dried (-50◦C) to obtain a powdered protein concentrate of *tobiko* filaments. The protein concentrate in the form of powder was then stored in the freezer.

Making Milk-Rice Corn

The process of making corn rice milk refers to soya milk processing.[23] Making rice-corn milk begins with washing rice and sweet corn with clean water. The washed rice is then roasted for 20 minutes until it dries and the distinctive aroma of rice can be smelled. The roasted rice is then soaked in hot water at a ratio of 1:2 overnight, followed by filtering to obtain rice with a softer texture. Sweet corn that has been washed and then boiled with water at a temperature of 85-90˚C for 15 minutes followed by shelling to obtain sweet corn seeds. The ready-to-process rice was then weighed according to the treatment, and each treatment was added with 10 g of sweet corn and added 200 mL of water followed by mixing in a blender. The results of the mixing are then filtered to obtain the filtrate. The filtrate was then boiled at a temperature of 70˚C ± 2 for 8-10 minutes by adding 12% sugar, 0.1% CMC(carboxymextyl cellulose), and protein concentrate powder.

Characterization Analysis

HPLC Analysis

HPLC analysis was carried out at IPB University Laboratory, Indonesia. The HPLC used was Thermo Scientific ODS-2 Hyersil, flow rate 1 mL/min, fluorescence detector, with Buffer A and Buffer B as mobile phases. Buffer A consisted of the above composition dissolved in 1 liter of water. Buffer B : consists of 95% methanol and water. Amino acid analysis : dissolve the hydrolyzed sample in 10 mL of 0.01N HCl then filter with milipore paper, add buffer potassium borate pH 10.4 with a ratio of 1: 1. Into a clean empty vial put 5 µl of sample and add 25 µl of OPA (o-Phthaldialdehyde Reagent Solution), let for 1 minute for complete derivatization. Inject into the HPLC column as much as 5 µl then wait until all the acid is separated.

Insoluble Part Analysis

The protein concentrate (sample, 2 g) were added hot water (20 mL), and than stirred it until dissolved. The solution was poured into filter paper that has been weighed. The filter paper was dried in an oven for 2 hours at 105˚C. The filter paper was placed in a desiccator for 15 minutes. Final weight was weighed of the mass of the insoluble precipitate divided by the initial mass times 100%.

Moisture and Ash Content Analysis

The sample was (2 g) was put in a cup, placed in an oven at 105˚C for 5 hours. After that, the cup is placed in a desiccator for 15 minutes. Final weight was weighed from the difference in mass obtained divided by the initial mass times 100%. The results of oven drying were then put into a furnace at a temperature of 500˚C for 8 hours. The results were then weighed by dividing the mass of ash divided by the mass of drying product multiplied by 100%.

Protein Content Analysis

Protein content analyzed with biuret method using BSA standard (Bovine Serum Albumine). Preparation of BSA standard solution : BSA (10 mg) were dissolved with aquadest into 10 mL volumetric flask. Preparation of standard curve solution : BSA standard solution were pipetted into a 5 mL volumetric flask (0 mL, 1 mL, 2 mL, 3 mL, and 4 mL), add 4 mL of aquadest (3 mL, 2 mL, 1 mL and 0 mL), and than add 1 mL of each biuret so that each concentration produces a total volume of 5 mL solution. From that step, the BSA standard were avalilable in concentrations : 0 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL and 1 mg/mL.Measurement of standad curve solution : The BSA solution (concentration : 0 mg/mL, 0.25 mg/mL, 0.5 mg/mL, 0.75 mg/mL and 1 mg/mL) were measured absorbance at a wavelength of 600 nm using spectroscopy UV-Vis (Shimadzu). From UV-Vis spectrum, the linear regression equation of the data obtained.Determination of protein content : protein concentrate (sample, 2 g) were crushed first and then dissolved in 100 mL of distilled water (sample concentration = 20 mg/mL), taken 3 mL and added 1 mL of 10% NaOH, 1 mL of Biuret reagent, then measured absorbance at a wave length of 600 nm (positive reaction marked purple). Calculation of protein content is carried out from the standard curve: $y = ax + b$ where x is the protein content and y is the absorbance of the sample.

Lipid Content Analysis

Lipid content was analyzed using fatty acid hydrolysis method. The sample (2 g) was added 4 mL of ethanol (96%) and 10 mL HCl solution (25 HCl: 11 aquadest). Erlenmeyer containing the sample was put in a water bath at a temperature of 70˚C for 30-40 minutes, then added 10 mL of 96% ethanol and cooled. The sample was added with 25 mL of petroleum benzene then homogenized gradually for 1 minute. The samples obtained were collected together and put into the in a separating funnel. In the separatory funnel, 2 layers will form in the form of a liquid, the layer used was the top layer. The liquid obtained wasthen baked for 30 minutes later weighed.Lipid content was determine from the mass of the lipid precipitate divided by the initial mass times 100%.

Carbohydrate Content Analysis

Carbohydrate analysis was done by difference method, as for the formula calculation as follows: Carbohydrates (%) = 100% – (moisture content + ash content + protein content + lipid content)

Energy Value Calculation

The determination of Nutritional Information is based on the Atwater Factor. The Atwater factor is the conversion rate of carbohydrates, fats, and proteins per gram in producing energy. For example, the Atwater factor for carbohydrates is 4 kcal/g, fat is 9 kcal/g, and protein is 4 kcal/g.

% Ingridients = (material weight (g)/ total raw material (g)) x 100%

Energy value
= Atwater factor x nutritional content in ingridients
= (4 kkal x carbohydrate content) + (9 kkal x lipid content) + (4 kkal x protein content)

Research Design

Protein extraction was carried out using four pH values, namely 7.5; 8; 8.5; and 9. The results of the three are then calculated the protein content. The highest protein content was then analyzed by HPLC to obtain its amino acid profile and applied to the manufacture of rice-corn milk. In the process of making rice-corn milk, a factorial randomized block design was used, namely the percentage of rice essence (5, 10, 15% v/v) and protein from *tobiko* filaments (0, 1, 2, 3% v/v) with a total volume of milk as much as 100 mL. As a result, the corn juice percentage was consistent during making process (5% v/v).

Result and Discussion

Amino Acid Profile of Protein Concentrate

The process of extracting protein from tobico filaments in this study used variations in alkaline pH at 7.5; 8; 8.5; 9 (based on isoelectric). The use of pH conditions at the time of protein extraction proved to have an effect on the structure, components, and functionality of a sample.[19] The results showed that the protein content was 49% (pH 7.5), 57% (pH 8), 73.5% (pH 8.5), and 58.5% (pH 9). Based on that result, it was chosen that pH 8.5 was the best pH for extracting protein from *tobiko* filaments.Protein content from the extraction at pH 8.5 is 73.5% (less than 90%) so the term extracted after this discussion uses the term protein concentrate.[24] The results of further analysis of the protein concentrate (pH 8.5) can be seen in Table 1.

Table 1: Protein Content, Yield, Insoluble Part of Protein Concentrate in pH 8.5

Parameter	Result
Protein Content	73.52 ± 0.07 %
Yield	9.04 ± 0.13 %
Insoluable Part	69.79 ± 0.02 %

The protein concentrate (pH 8.5) contains 73.52± 0.07% protein, the yield is 9.04± 0.13%, and the insoluble portion is 69.79± 0.02%. The protein content of the extraction under alkaline conditions (pH 8.5) from the results of this study supports previous studies that extraction under alkaline conditions is an effective method for the extraction of *tobiko* filaments. Previous studies used isopropanol, aquadest, and sodium hydroxide (NaOH) and produced extracts with a protein content of 72-74%.[9]

That extraction process also showed effectiveness in obtaining concentrates compared to without the extraction process. This is indicated by the comparison of the protein content of the protein concentrate (from this study) compared to the previous study which calculated the protein content of the raw material of *tobiko* filament (without the protein alkaline extraction process). Previous studies calculated the protein content of *tobiko* filaments at 33.7% on a wet based and 40.1% on a dry based10. The difference in protein content between protein concentrate (from this study, using pH 8.5) appeared to be higher (73.52%) compared to without the extraction process (33.7-40.1%)10.

The protein concentrate (pH 8.5) was then analyzed using HPLC to obtain a profile of the amino acids contained in it (Table 2).

Table 2: Amino Acid Profile of Protein Concentrate from Filamen Tobiko

Amino Acid	%w/w		
	Protein Concentrate (from this study, pH 8.5)	*Tobiko* Filament[10]	*Tobiko*[10]
Essential			
Threonine	2.86± 0.01	1,90 ± 0.01	1,00± 0.01
Valin	3.41± 0.02	2,83 ± 0.08	3,35 ± 0.06
Isoleucine	3.33 ± 0.01	1.63 ± 0.02	1.65 ± 0.01
Leucin	5.86 ± 0.01	2.52 ± 0.16	2.87 ± 0.07
Phenylalanine	2.30 ± 0.02	1.30 ± 0.09	1.21 ± 0.03
Histidine	1.38 ± 0.01	3.51 ± 0,09	0.89 ± 0.00
Licyin	3.69 ± 0.02	2.32 ± 0.11	0.98 ± 0.00
Methionine	1.21 ± 0.01	1.13 ± 0.03	1.59 ± 0.00
Non Essential			
Arginine	6.11 ± 0.01	2.56 ± 0.08	1.42 ± 0.13
Aspartic acid	3.75 ± 0.01	3.36 ± 0.02	2.55 ± 0.02
Serine	3.05 ± 0.02	2.50 ± 0.00	2.71 ± 0.07
Glycine	1.84 ± 0.01	2.25 ± 0.15	1.67 ± 0.03
Alanine	3.82 ± 0.01	3.21 ± 0.17	2.94 ± 0.10
Tyrosine	1.46 ± 0.01	1.71 ± 0.04	0.89 ± 0.02
Glutamate	7.08 ± 0.01	7.43 ± 0.17	5.38 ± 0.07

Table 2 shows the amino acid profile of the results of this study (protein concentrate from pH 8.5), compared to *tobiko* fillament[10] (starting material, without extraction process) and also *tobiko*[10] itself. The amino acid profile of the protein concentrate in this study showed the presence of essential amino acid (from higher to smaller percent) such as leucin (5.86 ± 0.01%), lycin (3.69 ± 0.02%), valin (3.41 ± 0.02%), isoleucine (3.33 ± 0.01%), threonine (2.86 ± 0.01%), phenylalanine (2.30 ± 0.02%), histidine (1.38 ± 0.01%), and methionine (1.21 ± 0.01%). Meanwhile, for non-essential amino acids from protein concentrate from this study showed (from higher to smaller percent) glutamate (7.08 ± 0.01%), arginine (6.11 ± 0.01%), alanine (3.82 ± 0.01%), aspartic acid (3.75 ± 0.01%), serine (3.05 ± 0.02%), glycine (1.84 ± 0.01%), and tyrosine (1.46 ± 0.01%).

Protein concentrate in this study has high amino acid content in glutamate, arginine, and leucine. Glutamate is an non essential amino acid that has an important role in nutrition, metabolism, and also acts as a neurotransmitter in a healthy brain.[25,26] Arginine is an essential amino acid that is useful for supporting fetal health in pregnant women, increasing reproduction, cardiovascular, lung, kidney, gastrointestinal, liver, immune, as well as facilitating wound healing, increasing insulin sensitivity, and maintaining tissue. In addition, arginine can be used for effective therapy for obesity, diabetes, and metabolic syndrome.[27-29] Leucine is an essential amino acid that is often used as a supplement or additive for the purpose of nutrification or enrichment in dietary foods.[30,31] This causes leucine to be used for the treatment of obesity as well as metabolic syndrome.[32]

Proximate Analysis of Nutrified Rice-Corn Milk

The protein concentrate of the tobico filament from the results of this study, was applied to non dairy-plant-based-milk from rice and corn. The addition is intended for nutrification, especially in increasing the protein content before and after being added. The results of the proximate analysis of nutrified rice-corn milk can be seen in Table 3.

Table 3: Proximate of Nutrified Rice-Corn Milk

Treatment	Proximate				
	Moisture Content (%)	**Ash Content (%)**	**Lipid Content (%)**	**Protein Content (%)**	**Carbohydrate Content (%)**
R_1C_0	72.42±0.02^e	0.23±0.02^a	0.09±0.02^a	12.53±0.02^a	14.74±0.02^e
R_1C_1	73.52±0.03^e	0.29±0.01^{ab}	0.10±0.03^b	15.31±0.02^d	10.78±0.01^b
R_1C^2	71,13±0.01^{de}	0.29±0.03^{ab}	0.13±0.03^{bc}	18.53±0.02^a	9.92±0.02^a
R_1C_3	73.55±0.03^e	0.23±0.02^a	0.19±0.01^a	14.34±0.02^c	11.70±0.03^c
R_2C_0	68.42±0.02^c	0.27±0.03^{ab}	0.10±0.02^a	13.06±0.02^b	18.14±0.01^f
R_2C_1	68.733±0.02^{cd}	0.35±0.02^c	0.17±0.03^a	18.06±0.02^g	12.70±0.02^d
R_2C_2	68.30±0.01^c	0.37±0.02^c	0.14±0.01^a	19.70±0.02^h	11.50±0.01^c
R_2C_3	69.30±0.03^{cd}	0.36±0.01^c	0.12±0.02^a	17.40±0.02^f	12.84±0.03^d
R_3C_0	67.90±0.01^{bc}	0.58±0.02^e	0.18±0.03^a	16.89±0.02^e	14.42±0.02^e
R_3C_1	69.80±0.02^{cd}	0.57±0.03^e	0.22±0.01^a	17.20±0.02^f	12.72±0.02^d
R_3C_2	65.70±0.01^{ab}	0.51±0.01^e	0.27±0.02^a	19.30±0.02^h	14.19±0.01^e
R_3C_3	65.07±0.02^a	0.50±0.01^d	0.281±0.02^a	21.18±0.02^i	12.95±0.02^d

The average value followed by the same letter shows no significant effect according to Duncan's test α = 1%

R_1C_0 (rice 5% : corn 5% : protein concentrate 0%)
R_1C_1 (rice 5% : corn 5% : protein concentrate 1%)
R_1C_2 (rice 10% : corn 5% : protein concentrate 2%)
R_1C_3 (rice 15% : corn 5% : protein concentrate 3%)
R_2C_0 (rice 5% : corn 5% : protein concentrate 0%)
R_2C_1 (rice 5% : corn 5% : protein concentrate 1%)
R_2C_2 (rice 10% : corn 5% : protein concentrate 2%)
R_2C_3 (rice 15% : corn 5% : protein concentrate 3%)
R_3C_0 (rice 5% : corn 5% : protein concentrate 0%)
R_3C_1 (rice 5% : corn 5% : protein concentrate 1%)
R_3C_2 (rice 10% : corn 5% : protein concentrate 2%)
R_3C_3 (rice 15% : corn 5% : protein concentrate 3%)

The moisture content in rice-corn milk ranges from 65.07-73.55%. The moisture content in cow's milk is 88.13-89.93%, while commercial rice milk products contain 89.28% water.[33] The interaction between starch and protein molecules that absorb water will cause the granules to swell and coincide with increasing the swelling power value.[34,35] The high swelling power can reduce the moisture content of the product and increase viscosity. The amylopectin content in rice tends not to quickly release the water absorbed due to hydrogen bonds 36,37. The ash content in rice-corn milk is about 0.23-0.58%. The ash content is not much different from previous studies using cereal seeds such as rice, peanuts, and soybeans, around 0.40-0.50%. Rice and corn contain calcium, magnesium, zinc, and iron.[38,39] Lipid content in rice-corn milk is between 0.09-0.27%. Lipid content increased with increasing rice content in the formulation. Lipid content in rice-corn milk is following previous research, which states that the maximum limit for lipid content in rice milk is 0.85%.[40]

Nutrification with the addition of protein concentrate from *tobiko* filaments in this study was proven to increase protein levels in rice-corn milk. Therefore, protein content in rice-corn milk (Table 3) was calculated as the average value based on the addition of *tobiko* filament protein concentrate (0, 1, 2, 3%), presented in Table 4.

Table 4: Average Protein Content of Rice-Corn Milk Products based on Protein Concentration Factor

Treatment	Average Protein Content (%) in 100 mL Rice-Corn Milk
Rice-Corn Milk without Protein Concentrate (0%)	14.17±0.02[a]
Rice-Corn Milk + Protein Concentration (1%)	16.86±0.01[b]
Rice-Corn Milk + Protein Concentration (2%)	17.64±0.02[c]
Rice-Corn Milk + Protein Concentration (3%)	19.19±0.03[d]

The average value followed by the same letter shows no significant effect according to Duncan's test α = 1%

Based on Table 4, it can be seen that the purpose of nutrification with the addition of *tobiko* filament protein concentrate was successful, with evidence increasing the average protein content on it (14.17-19.19% in a total of 100 mL). These results indicate that nutrified rice-corn milk are possible to be applied to children aged 1-3 years in accordance with the initial expectations of this study. It is related with nutritional adequacy rate for children aged 1-3 years, around 26 g/day.[41-43]

Table 5: Total Energy of Rice-Corn Milk

Treatment	Total Energy (kcal)	% Protein (Genaral Nutrition Requirement)
R1C0	219.77 ± 0.02	34.27 ± 0.01
R1C1	210.50 ± 0.07	41.87 ± 0.05
R1C2	229.97 ± 0.05	50.68 ± 0.02
R1C3	211.60 ± 0.03	39.22 ± 0.03
R2C0	251.43 ± 0.05	35.72 ± 0.01
R2C1	249.23 ± 0.07	49.40 ± 0.07
R2C2	252.20 ± 0.02	53.89 ± 0.04
R2C3	244.00 ± 0.07	47.58 ± 0.01
R3C0	253.70 ± 0.05	46.21 ± 0.02

R3C1	238.97 ± 0.02	47.04 ± 0.03
R3C2	272.73 ± 0.01	52.79 ± 0.02
R3C3	278.13 ± 0.03	57.93 ± 0.01

The average value followed by the same letter shows no significant effect according to Duncan's test α = 1%

R_1C_0 (rice 5% : corn 5% : protein concentrate 0%)
R_1C_1 (rice 5% : corn 5% : protein concentrate 1%)
R_1C_2 (rice 10% : corn 5% : protein concentrate 2%)
R_1C_3 (rice 15% : corn 5% : protein concentrate 3%)
R_2C_0 (rice 5% : corn 5% : protein concentrate 0%)
R_2C_1 (rice 5% : corn 5% : protein concentrate 1%)
R_2C_2 (rice 10% : corn 5% : protein concentrate 2%)
R_2C_3 (rice 15% : corn 5% : protein concentrate 3%)
R_3C_0 (rice 5% : corn 5% : protein concentrate 0%)
R_3C_1 (rice 5% : corn 5% : protein concentrate 1%)
R_3C_2 (rice 10% : corn 5% : protein concentrate 2%)
R_3C_3 (rice 15% : corn 5% : protein concentrate 3%)

Total Energy in Nutrified Rice-Corn Milk

The nutritional information obtained is an accumulation of the value of carbohydrates, proteins, and fats per 200 mL. Total energy of rice corn milk can be seen in Table 5.

The highest total energy gain was obtained from the R3C3 treatment (rice 15%: corn 5%: protein concentrate 3%), 278.13± 0.03 kcal. The total energy obtained is based on the composition of the ingredients used in the manufacture of rice-corn milk, namely rice, corn, sugar, and *tobiko* filament protein concentrate. Generally, fresh cow's milk and pasteurized milk products have a total energy of 42-61 kcal per 100 mL, while soy milk products have 44.2 kcal/100 mL and commercial rice-milk products have a total energy of 47 kcal/100 mL.[44] The total energy requirement for children aged 1 to 3 years is 1125 kcal, where the protein needs are 26 grams, fat 44 grams, and carbohydrates 155 grams per day 43. Low food intake during infancy will be irreversible (cannot be recovered), so that quality food intake is needed to support growth and development.[45,46]

Conlusion

Tobiko filament is a potential source of protein from marine products. This study showed that protein from tobico filaments can be extracted under alkaline conditions, according to the isoelectric point of the amino acids contained therein. The extraction result, protein concentrate, is proven to increase the protein content of non dairy-plant-based-milk from rice and corn. Further research are needed regarding consumer acceptance and product quality compared to commercial products.

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Conflict and Interest

The authors do not have any conflict of interest.

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