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Protein Content of Fish Waste and Fish Protein Powder from Clupeapallasii Valenciennes ("Tamban")

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Article Info
Volume 82
Page Number: 15455 - 15478
Publication Issue:
January-February 2020

Article History
Article Received*: 18 May 2019*
Revised*: 14 July 2019*
Accepted*: 22 December 2019*
Publication*: 28 February 2020*

Abstract
Fish waste, consisting of the head, bones, tail, and entrails, comprises of around 38% of the total fish material. The food processing industry uses only the flesh of the fish to produce their fish food products, and the fish wastes are often left unused, if not added as an ingredient to low-market-value products such as animal feeds or fertilizers. Improper disposal of fish waste can be hazardous to the surroundings where it is being disposed, particularly to other living things, because of the high biological oxygen demand in the biodegradation of these wastes, which would cause oxygen depletion in its disposal area. There is therefore a need to utilize fish wastes. One method is conversion of fish waste to fish protein powder (FPP). The resultant powder could be used as an additive in drugs, cosmetics, among other applications. For Clupeapallasii Valenciennes, known in the Philippines as tamban and is one of the known local alternatives for sardines, the properties of fish waste, including its protein content, have not been subject to much scientific study and is therefore inconclusive. This study determines the amount of protein in the head, bones, and viscera of C. pallasii and compares the amount to processed sardines. The results of this study could be useful in determining their possible uses in pharmaceutics and could help increase the economic value of fish waste. The fish wastes were blended into a homogenized fish paste and underwent acid hydrolysis in order to extract hydrolysate. This hydrolysate, which had a clear yellow color, underwent freeze-drying to convert it into powder. Biuret test colorimetry was used to determine the percentage of protein in the powder. The same method was utilized for unhydrolyzed fish paste for comparison on the amount of protein yielded. As a result, two averages were made. The average concentration of protein in fish protein powder was 38.45% ± 1.92 (mg/mL), while that of the homogenized (unhydrolyzed) fish paste was 25.01% ± 2.20(mg/mL). Both figures are significantly less than as is known in past studies on the amount of soluble protein in the flesh of sardines.

Keywords: *Acid Hydrolysis, Freeze-drying, Hydrolysis, Protein Hydrolysate*

1. Introduction

Background of the Study

Fish waste comprises parts of the body of the fish which are not commonly consumed by humans, including which are particles of head, bones, tails, and entrails. Fish waste is typically processed into low market-value products, such as animal

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feed, fishmeal and fertilizer, because of its high protein content (Hsu, 2010). However, a large fraction of the available fish waste is not being appropriately stored or managed, and is disposed in the ground or on the ocean, leaving them to biodegrade. The biodegradation of the organic components of the waste have a high biological oxygen demand and, if not managed properly, pose risks for the surroundings of the disposal area. If the wastes were thrown to the ocean, for example, the waste would cause reduced oxygen levels in the seawaters at the ocean bottom, smother living organisms in the ocean and perhaps introduce disease or invasive species to the ecosystem of the sea floor (Environmental Protection Agency, 2018).

An alternative method of using fish waste instead of just disposing it, is the recovery of protein from the waste through proteolysis. Proteolysis is the breaking down of protein into smaller peptides with the use of a strong acid or an enzyme. Moreover, decreasing the size of peptides from fish waste through hydrolysis makes it the most available amino acid source for various physiological functions of the human body (Neklyudov et al. 2000). The product of hydrolysis process is called fish protein hydrolysate (FPH). FPH is a substance produced from the hydrolysis of fish wastes in order to provide nutrients in forms that cells may easily utilize (Kristinsson & Rasco, 2000).

There are different types of hydrolysis based on the agent used to cleave the peptide bonds. First is the enzymatic hydrolysis where an enzyme is used to enhance bond cleavage in molecules with the addition of the elements of water. The other method is through acid hydrolysis. A protic acid is used to catalyze the cleavage of a chemical bond with the addition of the elements of water. However in the study of Bello &Generalao (2018), it is found that acid hydrolysis yields a greater amount of protein compared to enzymatic hydrolysis.

In this study, the FPH content produced from the fish wastes of "tamban" via acid hydrolysis using HCl was then freeze-dried to obtain the fish protein powder (FPP).

Objective of the Study

The main objective of this study is the production of fish protein powder (FPP) from the wastes of *Clupeapallasii*Valenciennes ("tamban"). Specifically,

1.) obtain fish protein power (FPP) via acid hydrolyses and subsequent freeze-drying,
2.) compare the protein content of the fresh fish waste to that of the fish protein powder.

Significance of the Study

Through hydrolysis, the waste components of "tamban" can be converted into a high-value product that is FPP, which can have a wide-range of applications in pharmaceutics, nutraceutics, cosmetics and many other industries, instead of just being converted into low-value products, like feeds and fertilizers.

Scope and Limitations of the Study

This study focuses on the production of the protein powder produced from *C.pallasii*via acid hydrolyses and freeze-drying. The acquired tamban was bought at Wet Market in Iligan City. Amino acid analysis and proximate analysis for the FPP are not included in the study.

Definition of Terms

Acid Hydrolysis – A type of hydrolysis that employs strong acid and is usually carried out at high temperature, as it attacks all peptide bonds in the protein substrate, to form amino acids.

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Freeze-drying – A low-temperature dehydration process that involves freezing the product, lowering pressure, then removing the ice by sublimation.

Hydrolysis – the process by which proteins are degraded into their component polypeptide or amino acid parts, generally occurs through protease-mediated hydrolysis of peptide bonds, and through non-enzymatic methods.

Protein Hydrolysate – a mixture of amino acids or peptides that is used as nutrient and fluid replenishers and prepared by splitting a protein with acid, alkali, or enzymes.

2. Methodology

Preparation of Sample

Procurement of the Fish Sample

One kilogram of the fresh fish sample *C.pallasii*(“tamban”) was procured from Wet Market, Iligan City, Philippines. The sample was then put in an icebox and transported to the Biology Laboratory of Philippine Science High School – Central Mindanao Campus. The fish was thoroughly washed and the flesh was separated from the bones, entrails, heads and tails, which compose the fish waste. The fish waste was put in the containers the weighed (Appendix A, Figure A.1).

Preparation of Homogenized Fish Paste

To make the homogenized fish paste, the amount of the fish waste was mixed with the same amount of distilled water in order to fulfill the 1:1 ratio of the waste and water, as recommended by the previous study of Generalao and Bello (2018). The formed mixture was then homogenized through utilization of a blender until all the components were uniformly mixed (Appendix A, Figure A.2). Then, the homogenized fish paste was divided into six bottles with each containing equal amounts of the paste.For comparison, two samples of fish pastes were made. Three of the six bottles were subjected to acid hydrolysis to obtain the FPH. The other three bottles of fish paste sample was used for protein content analysis right away (Appendix A, FigureA.4).

Preparation of Fish Protein Powder

Acid Hydrolysis

Before execution of the acid hydrolysis of C. pallasii, 12M of HCl was acquired from the school laboratory and was diluted to 4M. As adopted from Wisuthiphaet and Kongruang (2015), the fish parts were subjected to the optimum conditions for acid hydrolysis, namely the use of 1:1 ratio of 4M HCl to hydrolyse the same amount of homogenized paste under a pressure of 15 psi and temperature of 121°C for 90 minutes (Appendix B, Figure B.1). The three bottles with paste intended for acid hydrolysis were put inside the autoclave which was set to 121°C and 15 psi. Acid hydrolysis was performed for 90 minutes (Appendix B, Figure B.2). For the termination of hydrolysis, a few drops of 6M NaOH were added to the samples and were adjusted to pH 7.0 (Appendix B, Figure B.3).

Extraction of Fish Protein Hydrolysates (FPH)

After performing the acid hydrolysis, the samples were transferred from the container to 10mL centrifuge tubes, the samples were subjected to centrifugation with the settings at 5000 rpm for 15 minutes (Appendix C, Figure C.1), which was based on the study of Norma and NurAnati (2015). After

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centrifugation, layers were observed in the centrifuge tubes (Appendix C, Figure C.2). The identification of these layers were based on the research of Ramakrishnan, et al (Appendix C, Figure C.3). The protein layer was acquired by filtration using a filter paper (Appendix C, Figure C.4).

Freeze-drying

After obtaining the FPH, the sample was sent to Xavier University for freeze-drying. The product was frozen and subsequently dried in a vacuum chamber. The drying process was done in two phases: primary drying which removes frozen water through sublimation and secondary drying which removes non frozen bound water. Low temperature storage (−18°C) was done to preserve excellent functionality of freeze dried fish protein for up to 9 months (Carvajal et al. 2005).

Protein Analysis through Biuret Test/Colorimetry

The samples of homogenized fish paste and FPP were sent to Mindanao State University – Iligan Institute of Technology for protein analysis. Before determining the protein concentration of FPP, the spectrophotometer was calibrated by testing a protein standard in the spectrophotometer in order to infer the absorbance of protein at 540 nm at different standard concentrations, from which a line graph was created.

Analysis of Fish Protein Powder

The FPH was diluted and treated with an equal amount of 1% NaOH and $CuSO_4$. The treated FPH was subjected to colorimetric test in a spectrophotometer using UV/VIS spectroscopy at wavelength 540 nm. The absorbance of the sample, which pertains to the characteristic color of the mixture in spectroscopy, was recorded, and the concentration of protein in the sample was determined based on the calibration graph (Appendix E, Figure E.2).

Analysis of Homogenized Fish Paste

The FPH was diluted and treated with an equal amount of 1% NaOH and $CuSO_4$. The treated FPH was subjected to colorimetric test in a spectrophotometer using UV/VIS spectroscopy at wavelength 540 nm. The absorbance of the sample, which pertains to the characteristic color of the mixture in spectroscopy, was recorded, and the concentration of protein in the sample was determined based on the calibration graph (Appendix F, Figure F.2).

Data Analysis

The mean amount protein per unit measure of sample as well as percentage of protein by mass was measured. Data for the amount of protein per sample was presented as mean ± standard deviation. Single-factor analysis of variance (ANOVA) was used to compare the difference between the amounts of protein in fish protein powder and homogenized fish paste.

3. Results & Discussion

Preparation of Sample

After the separation of the meat of *C. pallasii* from the fish waste consisting of the head, bones, tail, and entrails, the sample subjected to acid hydrolysis to get the FPH had a total amount of 427.45 g of fish waste in one kilogram, while the sample whose resulting paste was subjected to immediate protein analysis had a total amount of 454.71 g of fish waste in one kilogram.

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After acid hydrolysis and centrifugation, the centrifuged fish paste formed layers. The soluble, clear protein layer, which was the FPH, was isolated through filtration and measured. The FPH was observed to have a clear yellow color and had a pungent odor. This FPH was then freeze-dried in order to convert it into FPP. The resulting FPP would then be analysed for protein content. For the sample that did not undergo acid hydrolysis, the homogenized fish paste was directly tested for protein content.

Protein Analysis

Biuret test/colorimetry was employed to estimate the protein content in the fish waste samples. To calibrate protein determination, the absorbance of protein under UV/VIS spectroscopy at 540 nm was measured from varying standard concentrations of protein. A line graph was then made to infer estimates of the concentration of protein based on the absorbance. Afterwards, the absorbance of the samples was measured, from which a concentration Cx was inferred. The Cx was multiplied with the dilution factor in order to determine the actual concentration of protein within the sample.

The fish protein powder was divided into 3 trials which were diluted in Biuret reagent. The first trial yielded an average of 39.98 mg of protein, the second 36.30 mg, and the third 39.06 mg. The average percentage of protein in the powder was 38.45% ± 1.92 (Table 1).

Table 1 Average concentration of protein in fish protein powder.

Sample	1	2	3	Average
Absorbance	0.1053	0.0956	0.1029	
Cx	0.3997	0.3629	0.3906	
Dilution factor	100	100	100	
Concentration of Protein (mg/mL)	**39.97 ± 0.69**	**36.29 ± 0.74**	**39.06 ± 0.80**	**38.45 ± 1.92**

The homogenized fish paste that did not undergo hydrolysis was divided into 3 trials

of 1 mL each which were treated with Biuret reagent. The first trial yielded an average concentration of 22.67 mg of protein, the second 24.05 mg, and the third 28.32 mg. The average percentage of protein in the paste was 25.01% ± 2.20 (Table 2).

Table 2 Average concentration of protein in fhomogenized fish paste.

Sample	1	2	3	Average
Absorbance	0.0467	0.0500	0.0603	
Cx	0.2267	0.2405	0.2832	
Dilution factor	100	100	100	
Concentration of Protein (mg/mL)	**22.67 ± 0.48**	**24.05 ± 1.09**	**28.32 ± 1.04**	**25.01 ± 2.20**

Based on the single-factor analysis of variance between the results of the protein analyses, it can be concluded that the fish protein powder yielded a significantly greater amount of protein per unit than homogenized fish paste (Table 3). Both concentrations, however, are greater than the concentrations of protein in most common food products, and the concentration of protein in homogenized fish paste is around just as much as that of canned American corned beef (Appendix G). It is also observed that the percentage of protein in FPP is greater than that of the average amount of protein found in canned sardines, although less than the amount in whole sardine muscle (Montero *et al*., 1998).

Table 3 Single-factor analysis of variance (ANOVA) between the means of the results from the protein analysis of fish

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protein powder (FPP) and homogenized fish paste

Group	Count	Sum	Average	Variance
FPP	3	115.34	38.4467	3.66773
Paste	3	75.04	25.0133	8.67663

Source of Variance	SS	df	MS	F	P-value	F critical
Between groups	270.682	1	38.4467	43.8551	0.0027	7.70865
Within groups	24.6887	4	25.0133			
Total	295.37	5				

Conclusion & Recommendations

Fish wastes consisting of the bones, head, viscera or internal organs from *C. pallasii* are found to contain a significant amount of protein which is comparable to the amount found in conventional canned sardines, although on average less than the amount in whole sardine muscle. Based on the single-factor analysis of variance between the results of the two samples of protein analyses, it can be concluded that the fish protein powder yielded a significantly greater amount of protein per unit than homogenized fish paste. The fish protein powder is therefore more feasible as a source of protein because other than its high protein concentration, FPP has a longer shelf life than either fish protein hydrolysate or fish paste.

This study can be furthered by performing other forms functional characterization such as proximate analysis, amino acid composition, gelling property, emulsifying property. Bioactive properties such as antioxidant and antibacterial properties can also be tested from the FPP produced in this study which can serve as antecedent for its applications in food technology and pharmacology. Also, other varieties of fish can be converted to FPP especially those with low-consumption rate.

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APPENDIX A

PREPARATION AND BLENDING OF FISH WASTE COMPONENTS

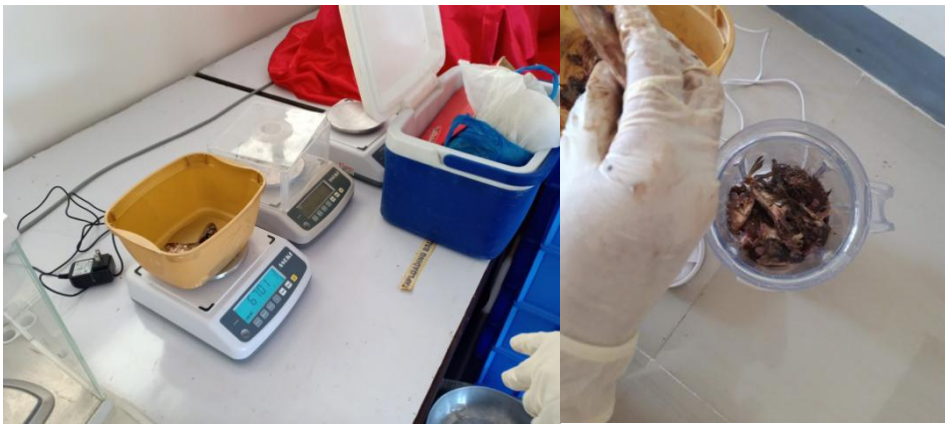

Figure A.1. Weighing and preparation of fish waste for homogenization (April 24, 2019 at PSHS-CMC Biology Laboratory)

Figure A.2. Blending of fish waste components with distilled water (April 24, 2019 at PSHS-CMC Biology Laboratory)

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Figure A.3. Weighing of the Fish Paste (April 24, 2019 at PSHS-CMC Biology Laboratory)

Figure A.4. Transferring of Fish Paste to Gatorade Bottles (April 24, 2019 at PSHS-CMC Biology Laboratory)

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APPENDIX B

ACID HYDROLYSIS

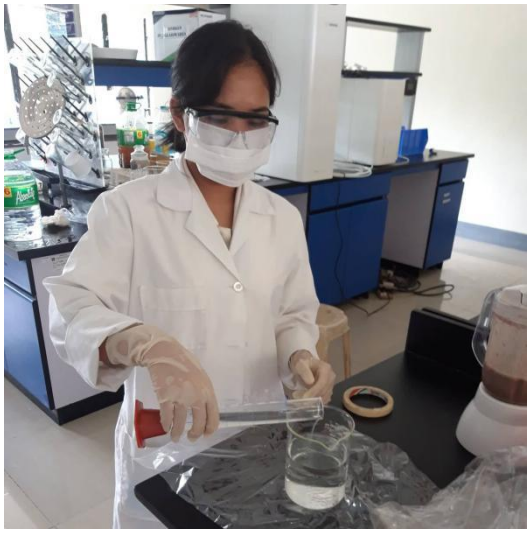

Figure B.1. Dilution of 12M HCl into 4M HCl concentration and application of 4M HCl into the fish waste solutions. (April 24, 2019 at PSHS-CMC Biology Laboratory)

Figure B.2. Performing of acid hydrolysis in an autoclave at 15 psi and 121 degrees Celsius for 90 minutes (April 24, 2019 at PSHS-CMC Biology Laboratory)

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APPENDIX C

ACQUIRING OF FISH PROTEIN HYDROLYSATE

Figure C.1. Adding of NaOH to the Fish Solution (April 24, 2019 at PSHS-CMC Biology Laboratory)

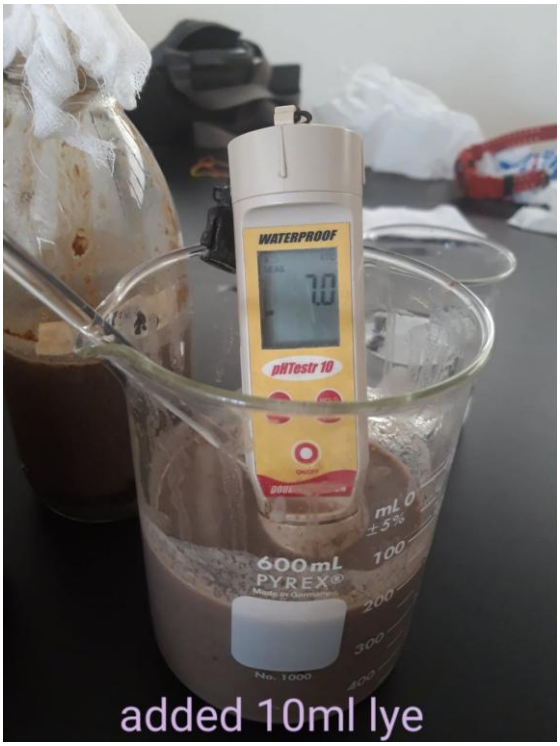

Figure C.2. Measuring the pH of hydrolyzed fish paste.

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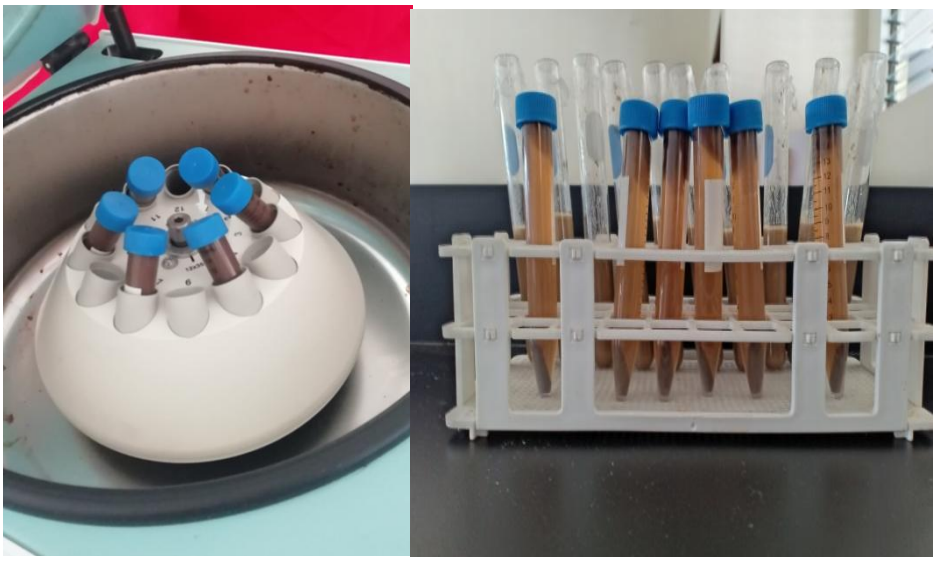

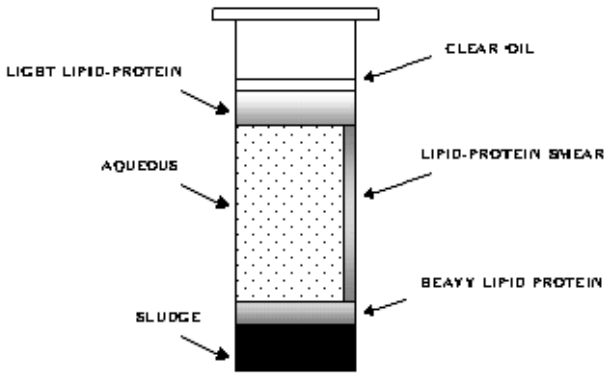

Figure C.3. Centrifugation of the fish solution at 5000 rpm for 15 minutes (April 24, 2019 at PSHS-CMC Biology Laboratory)

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Figure C.4. Filtration of the centrifuged fish samples to obtain the clear, yellow, soluble layer which is the FPH (February 28, 2019 at PSHS-CMC Biology Laboratory)

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APPENDIX D

PROTEIN ANALYSIS THROUGH BIURET TEST/COLORIMETRY

Figure D.1. Weighing of sample on September 7, 2019 at MSU-IIT

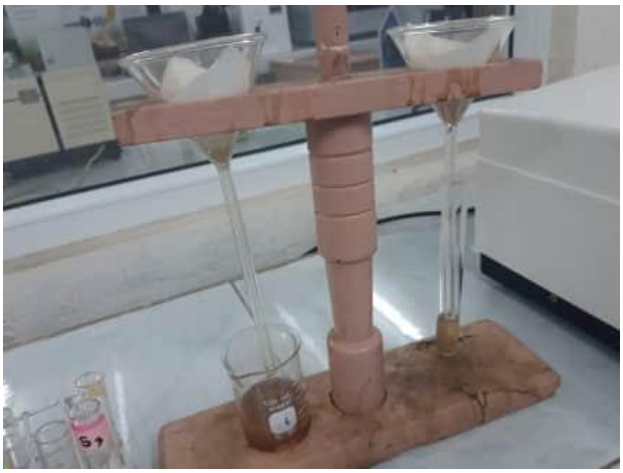

Figure D.2. Dilution of sample on September 7, 2019 at MSU-IIT

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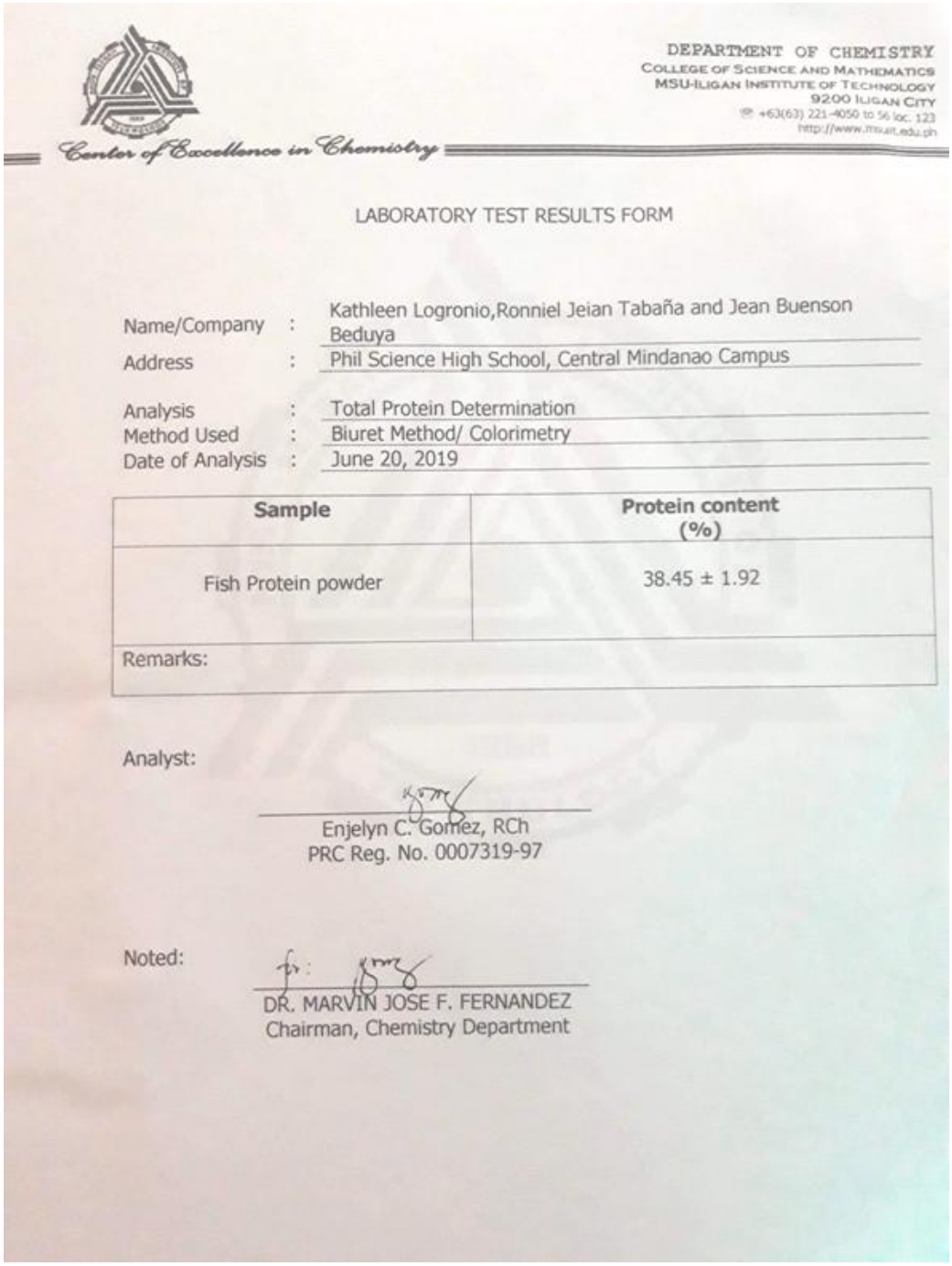
APPENDIX E

RESULTS OF PROTEIN ANALYSIS OF FISH PROTEIN POWDER



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Center of Excellence in Chemistry

LABORATORY TEST RESULTS FORM

Name/Company : Kathleen Logronio,Ronniel Jeian Tabaña and Jean Buenson Beduya
Address : Phil Science High School, Central Mindanao Campus

Analysis : Total Protein Determination
Method Used : Biuret Method/ Colorimetry
Date of Analysis : June 20, 2019

Sample	Protein content (%)
Fish Protein powder	38.45 ± 1.92
Remarks:	

Analyst:

Enjelyn C. Gomez, RCh
PRC Reg. No. 0007319-97

Noted:

DR. MARVIN JOSE F. FERNANDEZ
Chairman, Chemistry Department

Figure E.1. MSU-IIT Certification of protein content result

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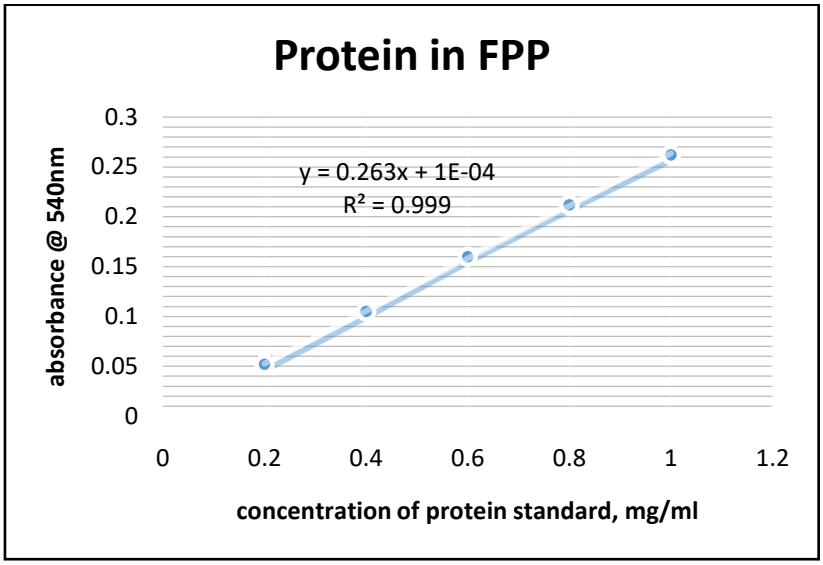

Figure E.2. Regression line graph after calibration of absorbance at 540 nm by concentration of protein standard in fish protein powder.

Table E.1. Concentration of protein in trial 1 of fish protein powder.

Trial 1 replicate	1	2	3	Average
Absorbance	0.1059	0.1026	0.1074	0.1053
Cx	0.4021	0.3894	0.4076	0.3997
Dilution factor	100	100	100	100
Concentration of Protein (mg/mL)	**40.21**	**38.94**	**40.76**	**39.97 ± 0.69**

Table E.2. Concentration of protein in trial 2 of fish protein powder.

Trial 2 replicate	1	2	3	Average
Absorbance	0.0986	0.0951	0.0932	0.0956
Cx	0.3741	0.3611	0.3538	0.3629
Dilution factor	100	100	100	100
Concentration of Protein (mg/mL)	**37.41**	**36.11**	**35.38**	**36.29 ± 0.74**

Table E.3. Concentration of protein in trial 3 of fish protein powder.

Trial 3 replicate	1	2	3	Average
Absorbance	0.1054	0.1010	0.1007	0.1029
Cx	0.4001	0.3834	0.3824	0.3906

Dilution factor	100	100	100	10
Concentration of Protein (mg/mL)	**40.01**	**38.34**	**38.24**	**39.06 ± 0.80**

Table E.4. Average concentration of protein in fish protein powder.

Sample	1	2	3	Average
Absorbance	0.1053	0.0956	0.1029	
Cx	0.3997	0.3629	0.3906	
Dilution factor	100	100	100	
Concentration of Protein (mg/mL)	**39.97 ± 0.69**	**36.29 ± 0.74**	**39.06 ± 0.80**	**38.45 ± 1.92**

APPENDIX F

RESULTS OF PROTEIN ANALYSIS OF HOMOGENIZED FISH PASTE

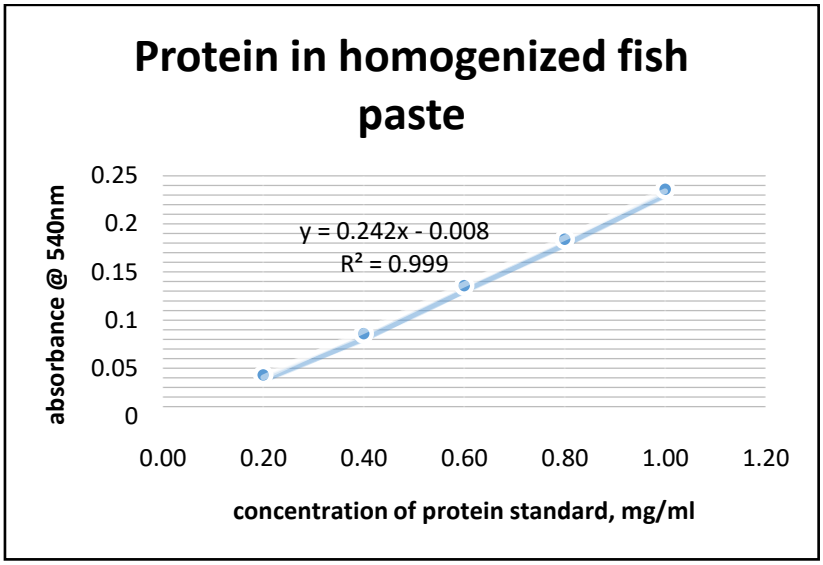

Figure F.1. Regression line graph after calibration of absorbance at 540 nm by concentration of protein standard in homogenized fish paste.

Table F.1. Concentration of protein in trial 1 of homogenized fish paste.

Trial 1 replicate	1	2	3	Average
Absorbance	0.048	0.046	0.046	0.0467
Cx	0.232	0.224	0.224	0.2267
Dilution factor	100	100	100	100
Concentration of Protein (mg/mL)	**23.223**	**22.397**	**22.397**	**22.67 ± 0.48**

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Table F.2. Concentration of protein in trial 2 of homogenized fish paste.

Trial 2 replicate	1	2	3	Average
Absorbance	0.049	0.053	0.048	0.0500
Cx	0.236	0.253	0.232	0.2405
Dilution factor	100	100	100	100
Concentration of Protein (mg/mL)	**23.636**	**25.829**	**23.223**	**24.05 ± 1.09**

Table F.3. Concentration of protein in trial 3 of homogenized fish paste.

Sample	1	2	3	Average
Absorbance	0.058	0.063	0.060	0.603
Cx	0.274	0.294	0.282	0.2832
Dilution factor	100	100	100	100
Concentration of Protein (mg/mL)	**27.355**	**29.421**	**28.182**	**28.32 ± 1.04**

Table F.4. Average concentration of protein in all trials of homogenized fish paste.

Sample	1	2	3	Average
Absorbance	0.0467	0.0500	0.0603	
Cx	0.2267	0.2405	0.2832	
Dilution factor	100	100	100	
Concentration of Protein (mg/mL)	**22.67 ± 0.48**	**24.05 ± 1.09**	**28.32 ± 1.04**	**25.01 ± 2.20**

APPENDIX G

PROTEIN CONTENT OF OTHER FOOD PRODUCTS

(Atayan et al, 2013)

Table G. The protein contents of different protein containing products in the market.

FOOD PRODUCT	AMOUNT OF SAMPLE (in grams)	Theoretical Content (% Protein) Min.	Theoretical Content (% Protein) Max.
Barley groats	1.5	6.70	10.50
Corn meal	1.5	6.72	10.00
Rice, polished	1.5	7.04	7.88

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Gray “sasing”	1.5	7.10	13.30
Sorghum	1.5	7.30	18.90
Rice, unpolished	1.5	7.56	7.88
Corn, whole grain	1.5	7.72	10.00
Peeled barley	1.5	7.80	12.00
Rye, whole grain	1.5	8.08	11.10
Buckwheat, peeled	1.5	8.12	10.03
Calf's brain	1.5	9.00	10.80
Barley, no peel, whole grain	1.5	9.70	11.30
Wheat, whole grain	1.5	10.02	12.98
Ox brain	1.5	10.20	10.70
Frying sausage from veal	1.5	10.30	12.00
Pig's brain	1.5	10.60	10.60
Sausage 'Galbwurst'	1.5	11.30	11.30
Oat groats	1.5	11.40	16.30
Spelt	1.5	11.50	11.50
Sausage 'Lyoner'	1.5	11.50	11.50
Frying sausage from pork	1.5	11.50	11.50
Sausage 'Munich Weißwurst'	1.5	11.60	11.60
Oats, no peel, whole grain	1.5	11.90	13.20
Pig's lung (lights)	1.5	11.90	14.90
Sausages canned	1.5	11.90	14.10
Oat meal	1.5	12.00	14.40
Mutton, brisket	1.5	12.00	12.00
Sheep's tongue	1.5	12.00	14.60
Sausage 'Flaischwurst'	1.5	12.10	12.10
Pink “sasing”	1.5	12.20	16.00
Meatloaf	1.5	12.40	12.40

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Vienna sausages	1.5	12.40	12.40
Calf's tripe (offal pluck)	1.5	12.70	16.90
Liver sausage 'homemade'	1.5	12.70	12.70
Brown “sasing”	1.5	12.90	15.80
Pig's tongue	1.5	12.90	16.40
Triticale	1.5	13.13	14.35
Brawn, red	1.5	13.20	13.20
Sausage 'Schinkenwurst', delicate	1.5	13.50	13.50
Mutton, shoulder	1.5	13.69	13.69
Luncheon meat	1.5	13.80	15.30
Calf, neck sweet bread	1.5	14.00	19.60
Brawn	1.5	14.70	14.70
Mutton, chop	1.5	14.90	14.90
Calf's heart	1.5	15.00	17.20
Calf's kidney	1.5	15.00	18.00
Ox kidney	1.5	15.00	17.70
Brawn, white	1.5	15.10	15.10
Chicken, heart	1.5	15.40	20.50
Amarant, seeds	1.5	15.58	15.93
Ox lungs (lights)	1.5	15.60	20.10
Sheep's kidney	1.5	15.70	18.00
Ox tongue	1.5	15.70	16.40
Duck, average	1.5	16.00	20.80
Mutton, sirloin	1.5	16.20	20.20
Veal, brisket	1.5	16.30	20.80
Calf's tongue	1.5	16.30	17.90
Pig's kidney	1.5	16.30	17.00
Sausage 'Bierwurst'	1.5	16.30	16.30
Sausage 'Jagdwurst'	1.5	16.30	16.30

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Ox heart	1.5	16.50	16.90
Calf's lungs (lights)	1.5	16.60	18.30
Sheep's lungs (lights)	1.5	16.70	21.20
Sheep's heart	1.5	16.80	16.80
Sausage 'Gattinger'	1.5	16.80	16.80
Pig's heart	1.5	16.90	16.90
Ox blood	1.5	17.00	18.10
Pig's spleen	1.5	17.10	17.60
Sheep's spleen	1.5	17.20	18.80
Ox spleen	1.5	17.40	21.90
Chicken, (boiling fowl), average	1.5	17.40	19.00
Corned beef, German	1.5	17.50	25.10
Pork, shoulder with skin (blade of shoulder)	1.5	17.55	17.55
Calf's liver	1.5	17.70	20.60
Beef, canned	1.5	17.80	19.20
Sausage 'Bierschinken'	1.5	17.80	17.80
Mutton, leg	1.5	18.00	18.00
Pig's blood	1.5	18.00	19.20
Goat meat, average	1.5	18.00	20.70
Calf's spleen	1.5	18.10	18.30
Horse meat, average	1.5	18.10	21.70
Venison, average	1.5	18.10	22.20
Pork, chuck	1.5	18.30	18.30
Beef, brisket	1.5	18.35	18.35
Turkey, adult animal, average, with skin	1.5	18.40	21.00
Mutton, muscle only	1.5	18.50	21.30
Pork, loin, cured (Kassel)	1.5	18.50	22.20

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Chicken, leg with skin, without bone	1.5	18.50	18.50
Pork, hip bone (hind leg)	1.5	18.95	18.95
Beef, top round	1.5	19.10	22.00
Beef, chuck	1.5	19.25	19.25
Pig's liver	1.5	19.50	21.30
Wild boar meat, average	1.5	19.50	19.50
Ox liver	1.5	19.70	20.60
Turkey, leg, without skin and bone	1.5	19.80	21.10
Ham, canned	1.5	19.90	20.50
Black pudding, type Thüringer'	1.5	19.90	19.90
Chicken, (chicken for roasting), average	1.5	19.90	19.90
Hare, average	1.5	20.00	23.00
Beef, shoulder	1.5	20.20	20.20
Rabbit meat, average with bone	1.5	20.30	21.10
Minced meat (steak tartar)	1.5	20.50	21.80
Beef, fore ribe, entrecote (roast joint)	1.5	20.55	20.55
Veal, fillet	1.5	20.60	20.60
Beef, muscles only	1.5	20.60	22.70
Veal, leg of veal with bone	1.5	20.70	20.70
Lamb, muscles only	1.5	20.80	20.80
Veal, shoulder	1.5	20.90	20.90
Veal, chop, cutlet with bone	1.5	20.90	20.90
Pigeon, average, with skin and bone	1.5	20.90	20.90
Sheep's liver	1.5	21.00	21.70

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Veal, knuckle with bone	1.5	21.00	21.00
Veal, neck with bone	1.5	21.20	21.20
Beef, fillet	1.5	21.20	21.20
Venison, haunch (leg)	1.5	21.20	21.70
Veal, muscles only	1.5	21.30	21.30
Beef, rump	1.5	21.45	21.45
Pork, chop with bone	1.5	21.60	21.60
Venison, back	1.5	21.70	23.40
Beef, silverside	1.5	21.75	21.75
Pork, fillet	1.5	22.00	22.00
Chicken, liver	1.5	22.10	22.10
Quail, average, without skin and bone	1.5	22.15	22.60
Pork, leg (hind leg)	1.5	22.20	22.20
Chicken, breast with skin	1.5	22.20	22.20
Turkey, breast without skin	1.5	22.40	25.20
Beef, roast beef (sirloin)	1.5	22.45	22.45
Ham, cooked	1.5	22.50	22.50
Pheasant, average (with skin, without bone)	1.5	22.70	24.80
Beef, minced meat	1.5	22.90	22.90
Corned beef, American	1.5	25.30	25.30