Marine Science Center-University of Basrah

Mesopotamian Journal of Marine Sciences
Print ISSN: 2073-6428
E- ISSN: 2708-6097
www.mjms.uobasrah.edu.iq/index.php/mms

Preparing fish protein concentrate from ray fish by water and alkaline hydrolysis and their physiochemical and microbial properties

iD Ahmed Sh. Al-Hassoon*, iD Qusay H. Al-Hamadany and iD Ameer A. Mohammed
Marine Science Center, University of Basrah, Basrah-Iraq
*Corresponding Author *:e-mail: ahmed999alhassoon@gmail.com*

Article info.
✓ *Received: 1 March 2021*
✓ *Accepted: 3 June 2021*
✓ *Published: 29 June2021*

Key Words:
Alkali hydrolysis
Distilled water
Physiochemical properties
Protein concentrate
Ray fish

Abstract - The present study aims to produce protein concentrate from Ray fish Himantura randalli. Fish samples were brought from the Iraqi seawater (Fao region), two methods of hydrolysis were used, the first with distilled water and the second with alkali solution (NaOH 0.1N) to recover protein concentrates from fish samples. The yield of protein concentrates recovered by distilled water and alkali solution were 7.2% and 10.1%, respectively, the protein content in the produced concentrates were 82.33% and 84.25% and the fat contents were 6.05% and 7.12% in the mentioned methods, respectively. The study has also tested the functional properties of the produced concentrates, (Solubility, water absorption and fat bending), the measurements of these properties were 60.8%, 67.1%; 3, 2.5 ml/gm and 3.5, 3 ml/gm for the two produced concentrates, respectively. The two concentrates have brown color with light fishy smell and showed good storage ability after 60 days under 28 °C and 7 °C without changes in color or smell, it also showed low total bacterial counts which were 429, 386 CFU/gm and 368, 357 CFU/gm for the two concentrates, respectively.

انتاج مركزات بروتينية من أسماك اللخم باستخدام طريقتي التحلل المائي والقاعدي ودراسة خصائصها الفيزيوكيميائية والميكروبية

أحمد شهاب الحسون، قصي حامد الحمداني و أمير عباس محمد
مركز علوم البحار، جامعة البصرة، العراق

المستخلص - تهدف الدراسة الحالية الى إنتاج مركز بروتيني من أسماك اللخم البحرية *Himantura randalli*، جلبت عينات الأسماك من المياه البحرية العراقية (منطقة الفاو). استخدمت طريقتان في إنتاج المركز البروتيني، الأولى باستخدام التحلل بالماء المقطر والثانية باستخدام المحلول القاعدي (NaOH عياري 0.1) لاستخلاص المركز البروتيني من عينات الأسماك، كان الحاصل من المركز البروتيني المستخلص بالطريقتين أعلاه 7.2% و 10.1% على التوالي. اختبرت الصفات الوظيفية للمركزين البروتينيين المنتجين (الذائبية، امتصاص الماء وربط الدهن)، إذ كانت قيم هذه الصفات 60.8% و 67.1% و 3 و2.5 مل/غم، 3.5 و3 مل/غم للمركزين المنتجين على التوالي. اتصف لون المركزين المنتجين باللون البني مع رائحة سمكية خفيفة وأظهرا قابلية خزن جيدة بعد فترة خزن 60 يوماً بدرجة حرارة 28 ºم و7 ºم وبدون تغير باللون والرائحة، كذلك أظهر المركزان المنتجان انخفاضاً في قيم العد البكتيري الكلي في درجتي الحرارة أعلاه إذ كانت 429 و 386 وحدة قياس المستعمرات/غم و 368 و 357 وحدة قياس المستعمرات/غم للمركزين المنتجين على التوالي.

الكلمات المفتاحية: مركز بروتيني، اسماك اللخم، التحلل بالماء المقطر، التحلل بالقاعدة، الخصائص الفيزيوكيميائية.

Introduction

The Arabian Gulf is considered as one of the richest areas in various shark fishes, but this resource is not invested at the present time despite that it is formed high percentage of demersal fish, this is because it is not consumed by most people in this region, and there is no ability for manufacturing these fishes to fish meal or for exportation (Eldahham, 1977).

Fish meal is actually a major source of protein in fish diets because it is a good source of essential amino acids (Xue *et al*., 2001 and Samocha *et al*., 2004). Fish can't create the ten essential amino acids especially Lysine and Methionine which are the first integrated amino acids, so it must be supplemented in fish diets (Craig and Helfrich, 2002).

Fish culture is the most developing food production system in the world, this fast developing greatly depends on increasing in fish diets production that mostly contain fish meal as a main resource of protein (Hardy and Barrows, 2002).

DOI: https://doi.org/10.58629/ mjms.v36i1.16.,

The global production of fishmeal is between 6-7 million t/y, this continuous increasing of fishmeal demand depends on using it in animal feeding especially in fish nutrition, that causes in creasing in demand and prices (FAO, 2001).

The demands on fish meal used in global aquaculture reached 32% of total global production in 1999 (New and Wijkstom, 2002), and 37% in 2000, and reached 70% in 2015, (Chamberlain, 2000), as a result of lasting and growing use of fish meal in fish food processing in addition to it's limited resources it becomes more costly (FAO, 2004).

It is good to know some functional properties of fish protein concentrates (FPC), it is identified as wide physical attitude or what protein achieved in food that reflects the reactions that occurred and affected by its structures and composition (Richardson, 1977 and Jasim, 1983).

The functional properties correlated with the protein and make it one of the useful food components and ingredients are: color, flavor, staple, softness, roughness, solubility, swelling, water pounding, glutinous, emulsification foaming, elasticity, adhering and fiber forming (Wilding *et al.*, 1984; Dalaly and Al-Rekaby, 1988).

The aim of this study is to produce two kinds of FPC via two methods, the first by using alkaline solution, and the other by using distilled water.

Materials and Methods

The Fish Protein Concentrates (FPC):

The FPC was prepared from fresh Ray fish (using the entire body of the fish) via two methods, the first by using alkaline solution, and the second by using distilled water; according to the procedures mentioned by Al-Taee (1986).

Preparing the FPC via Alkaline Hydrolysis:

The fish samples were mixed in a mixer after adding distilled water at a rate of 1:1.5 (weight). NaOH solution (0.1N) was added till the pH reached 10.5. Alkaline hydrolysis continued for one hour with stirring and keeping the pH stable at 10.5. The solution was centrifuged at 4000 c/for 20 minutes. The filtered solution was precipitated by adding HCl (0.1N) to drop the pH to 5.3, then centrifuged at 4000 c/m for 20 minutes to get a precipitate protein concentrate which dried by vacuum oven at 60 °C, then grinded and put in clean containers and kept in the refrigerator at (-17 °C).

Preparing the FPC via Water Hydrolysis:

A measured weight of row material was mixed in a mixer with distilled water at a rate of 1:1.5, and then kept for one hour for hydrolysis. The concentrate was filtered by using rotary vacuum evaporator at 40 °C, and then the protein concentrate was dried by vacuum oven on 60 °C, then grinded and put in clean containers and kept in the refrigerator at (-17 °C).

Yield:

The yield of the prepared FPC measured on the basis of the percentage weight of the obtained product divided by the weight of the raw materials.

Chemical Composition:

The chemical composition (protein, lipid, ash and the yield ratio) of the dried Ray fish and FPC were estimated according to the procedures mentioned by Al-Taee (1986).

Functional Properties:

Fat Binding:

The protein ability for fat binding was estimated by using the method of Souissi *et al*. (2007), by mixing 0.25 gm of the sample with 10 ml fat in a stirrer for 30 seconds and the sample was kept at the lab temperature for 10 minutes, then the precipitate was centrifuged at 4300 c/m for 10 minutes, the filtrate was put in a cylinder and the amount of the absorbed fat in the sample was measured by subtracting the amount of fat in the cylinder from the total fat amount.

Solubility:

The protein solubility was estimated by using the method of Taheri *et al* (2013), by mixing 0.25 gm of the sample with 20 ml distilled water by a stirrer and the sample was kept for 30 minutes at the lab temperature, then centrifuged at 4000 c/m for 30 minutes, after that, the sample was mixed with NaOH (0.5 N) and the solubility estimated as shown:

$$\text{Solubility } (\%) = \frac{\text{Soluble Protein Content}}{\text{Total Protein Content in the Sample}} \times 100 \text{-------(1)}$$

Water Absorption:

The protein ability for water absorption was estimated by using the method of Taunkara *et al* (2013), by mixing 0.25 gm from the sample with 10 ml distilled water in a stirrer for 30 seconds and the sample was kept at the lab temperature for 10 minutes, then precipitated with centrifugation at 4300 c/m for10 minutes, the filtrate was put in a cylinder and the amount of the absorbed water in the sample was measured by subtracting the amount of water in the cylinder from the total water amount.

Total Bacterial Counts:

The forementioned method was followed (A.P.H.A., 1984), in estimating the total bacterial counts. The total bacterial count was estimated for two protein concentrates separately, 1 gm of the sample was transferred to a dilution tube containing 9 ml of sterile peptone water, from which the decimal dilution was performed, and then 1 ml of appropriate dilution was transferred into sterilized empty dish, and 15 ml of nutrient medium were added, mixed well and gently and left until solidification occurred. Then the dishes were placed in the incubator upside down at a temperature of 37 °C for 48 hours, the total number of bacteria was calculated as follows:

$$\begin{aligned}(\text{Total number of Bacteria (g/cell)} = \text{Average Number of Colonies} \\ \times \text{Reciprocal of the Dilution Used}) - - - -(2)\end{aligned}$$

Statistical Analysis:

The statistical program SPSS (SPSS, 2000) was used in a comparison between the two concentrates produced by distilled water and alkaline and yield rate.

Results

Table (1) shows the raw material (Ray fish) chemical composition rates. Proteins were 52.05%, fats 20.61%, ashes 9.80% and moisture 6.83%. Table (2) shows the chemical composition rates of the protein concentrates prepared by water hydrolysis and alkaline hydrolysis and yield rate. The proteins, fats, ashes and moisture rates for the protein concentrate prepared by water hydrolysis were 82.33%, 6.05%, 6.67% and 3.78%, respectively, and the yield rate was 7.2%. While the rates of the mentioned components for the protein

concentrate prepared by alkaline (0.1 N) were 84.25%, 7.12%, 3.41% and 4.13%, respectively, and the rate of the yield was 10.1%.

Table 1. Chemical composition of Ray fish (Dry weight).

Material	Protein	Fat	Ash	Moisture
Ray fish	52.09	20.61	9.80	6.83

Table 2. Chemical composition of FPC and yield rate.

Item	Concentrate produced by distilled water	Concentrate produced by alkaline
Protein (%)	82.33 ± 0.11 a*	84.25 ± 0.19 a
Fat (%)	6.05 ± 0.22 a	7.12 ± 0.18 a
Ash (%)	6.67 ± 0.12 a	3.41 ± 0.28 b
Moisture (%)	3.78 ± 0.14 a	4.13 ± 0.22 a
Yield (%)	7.2 ± 0.34 a	10.1 ± 0.15 b

*Different letters of the same adjective indicate the presence of significant differences at the level ($p < 0.05$).

Table (3) shows the values of some estimated functional properties, the values of solubility, water absorption and lipid binding for the produced concentrate by distilled water were 60.8%, 3 ml/gm and 3.5% ml/gm, respectively, while for the concentrate produced by alkaline were 67.1%, 2.5 ml/gm and 3 ml/gm, respectively.

Table 3. Functional properties of FPC.

Sample	Functional Properties		
	Solubility (%)	Water Absorption (ml/gm)	Fat Binding (ml/gm)
Concentrate produced by distilled water	60.8	3	3.5
Concentrate produced by alkaline	67.1	2.5	3

Table (4) shows the bacterial number for the two concentrates during the storage period for 60 days at temperatures 28 °C and 7 °C, were 429 and 386 CFU/gm respectively for the produced concentrate by distill water, while they were 368 and 357 CFU/gm, respectively for the concentrate produced by alkaline hydrolysis.

Table 4. Total Plate Count of FPC during Storage (CFU/gm).

Sample	Temperature (C^o)	Storage Period (days)		
		0	30	60
Concentrate produced by distilled water	28	334	389	429
	7	334	358	386
Concentrate produced by alkaline	28	311	342	368
	7	311	334	357

Discussion

Table (1) shows the chemical composition of the raw material (Ray fish) and the percentage of produced concentrate by distilled water and alkaline hydrolysis and the yield rate. Table (2) shows an increase in the protein rates in the produced concentrates compared with the raw material, this refers to hydrolysis rate in the two used methods, the protein percentage in both produced concentrates were higher compared to what Mohammad and Al-Serajy (2013) found, when they produced protein concentrate from some fish viscera by using enzymatic hydrolysis, as the protein percentage was 70.2%, it was also higher than that Ali obtained from poultry by products by using both methods of the study (Ali, 2002).

The protein percentage in both produced concentrates were 79.84% and 80.82%, respectively, this refers to the recovery of part of myofibrils proteins that forms 75% of the total proteins. The yield rate of the produced protein concentrate by alkaline was 10.1%, it is higher compared to what Mohammad and Al-Serajy (2013) found from common carp viscera by using Papain enzyme, the yield was 7.6%, this difference refers to the hydrolysis rate of both methods used, while protein percentages of the produced protein concentrates by the two methods in this study was higher than that obtained by Maskat *et al*. (2010), as they got 65%. A statistical analysis of the components of the chemical composition of the two prepared protein concentrates was conducted, as no significant differences were found ($p>0.05$) for proteins, fats and moisture between the two FPC, while there was a significant difference ($p<0.05$) for ashes between the two FPC.

The statistical analysis also showed that there was a significant difference ($p<0.05$) in the yield between the two FPC. Some of the functional properties measured were the solubility, it was 60.8% by using distilled water, that was approximate to what Mohammad *et al*. (2013) found by enzymatic hydrolysis, it was 61.5%, while it was higher by alkaline method, it was 67.1%, this refers to the increasing in solubility according to protein breaking to small units (peptides), (Shahidi, 1994).

Water absorption in the produced protein concentrate by distilled water was 3ml/gm, it was higher compared to what Mohammad and Al-Serajy (2013) found at pH 10, (2.5 ml/gm), this agreed with the water absorption by alkaline method in this study, and also with what Autio *et al*. (1984) found.

The physical properties of both concentrates produced in this study shows that they have brown color and light fish smell varied from the result of Mohammad and Al-Serajy (2013), the produced protein concentrate by enzymatic hydrolysis characterized by yellow color and acceptable fish smell.

Table (4) shows the total bacterial counts of the prepared FPC, since they were less than the permissible limit according to ISo (2013), which is 10^3 CFU/gm, this indicates that they are within the health permissible limits to be added to the animal diets.

Conclusion

From the results of the current study, the following can be concluded:

1. Fish protein concentrates can be produced using both methods used in commercial quantities, as they are low in cost and contribute to support the production of animal feed.
2. It has good storage capacity as the bacterial counts is much less than the global permissible limit.

References

Ali, H.I. 2002. Producing protein concentrates from poultry by product and studying its chemical composition and functional properties. M.Sc. Thesis, Agriculture College, University of Basrah, 79 p.

Al-Taee, M.A.J. 1987. Meat and Fish Technology. University of Basrah Press, 421 p. (In Arabic).

A.P.H.A. (American Public Health Association). 1984. Recommended methods for the microbiological examination of foods. 2nd ed. M.L. Speck (ed.), Washigton D.C.

Autio, K., Kiesvaara, M., Malkki, Y. and Kanko, S. 1984. Chemical and functional properties of blood globin prepared by a new method. J. food Sci., 49: 859-862.https://doi.org/10.1111/j.1365-2621.1984.tb13228.x

Chamberlain, G.W. 2000. Aquaculture projections for use of fishmeal and oil. Oral presentation at the Annual Meeting of IFOMA, Lima, Peru, 30 October–3. https://scholar.google.com/scholar?cluster=10881847028819582907&hl=ar&as_sdt=2005&sciodt=0,5.

Craig, S. and Helfrich, L.A. 2017. Understanding fish nutrition, feeds and feeding, Virginia Cooperation Extension, Virginia State University, Department of Fisheries and Wildlife Sciences, Virginia Tech., p: 220-256. https://vtechworks.lib.vt.edu/bitstream/handle/10919/80712/FST-269.pdf.

Dalaly, B.K. and Al-Rekaby, K.H. 1988. Food Chemistry. Ministry of Higher Education and Scientific Research, Directorate of book House for Press and Publication, University of Mosel.

El-Dahham, N. 1977. Fish of Iraq and Arabian Gulf. Volume (1), publications of Arabian Gulf Studies Center, University of Basrah. Alrashad Press, Baghdad, 546 p. (In Arabic).

FAO. cbnm. 2001.Fishery Statistics Commodities .Vol.93, 226 p. https://www.fao.org/fishery/en/publication/71056.

FAO. State of World Fisheries and Aquaculture. 2004. FAO, Rome, Italy. 153 p. https://www.fao.org/documents/card/es/c/39161535-bb34-506e-a5ae-58aa68282435/.

Hardy, R.W. & Barrows, F.T. 2002.Diet formulation and manufacture .In: Fish Nutrition, 3rd ed. (Halver, J.E. & Hardy, R.W. eds.), Academic Press Inc., San Diego CA.USA. pp: 506-601. https://www.elsevier.com/books/fish-nutrition/hardy/978-0-12-319652-1.

ISO 2013. Microbiology of food and animals feeding stuffs-UN EN ISO 4833-1 Horizontal method for enumeration of microorganisms. Part 1: Colony count at 30 degrees C by the pour plate technique. ISO Norm 4833-1: 2013. International Standardization Organization ed., Geneva Switzerland. https://www.iso.org/obp/ui/#iso:std:iso:4833:-1:ed-1:v1:en.

Jasim, M.A. 1983. Functional plastein from fish waste. Ph.D. Thesis, Lough Borough University of technology, England. 294p. https://repository.lboro.ac.uk/articles/thesis/Functional_plastein_from_fish_waste/9238337.

Maskat, M.Y., Haslaniza, H., Wan Aida, W.M. and Mamot, S. 2010. The effects of enzyme concentration, temperature and incubation time on nitrogen content and degree of hydrolysis of protein precipitate from cockle *Anadara granosa* meat wash water. International Food Research Journal, 17: 147-152. https://myjurnal.mohe.gov.my/public/article-view.php?id=12514.

Mohammad, A.A. and Al-Serajy, A.Y. 2013. Studies on some functional properties of common carp (*Cyprinus carpio* L.) viscera protein concentrate by using papain. Basrah J. Agric. Sci., 26(1): 100-109. (In Arabic). https://www.iasj.net/iasj/article/111316.

New, M.B. and Wijkstom, U.N. 2002. Use of fish meal and fish oil in aquafeeds: Further thoughts on the fish meal trap. FAO Fisheries Circular No. 975 FIPP/C975. Food and Agriculture Organization of the United Nations, Rome. Properties of *Sardinella aurita* By-Product Hydrolysates, Food. Technol. Biotechnol., 45(2): 187-194. https://agris.fao.org/agris-search/search.do?recordID=XF2003413425.

Richardson, T. 1977. Functionality changes in proteins following action of enzymes. In: "Food proteins". Feeney and Whitaker, Washington, Dc. 458 p.DOI: 10.1021/ba-1977-0160.ch007

Samocha, T.M., Davis, D.A., Saoud, I.P. and DeBault, K. 2004. Substitution of fish meal by co-extruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 231: 197-203.https://doi.org/10.1016/j.aquaculture.2003.08.023

Shahidi, F. 1994. Sea food processing by-products. In: Seafood: Chemistry, Processing Technology and Quality, F. Shahidi, J.R. Botta (Eds.), Blackie Academic and Professional, London, UK, pp: 321-334.https://doi.org/10.1007/978-1-4615-2181-5_16

SPSS, 2000. SPSS for windows base system users guide, release 10.0 Chicago, USA.

Souissi, N., Bougatef, A., Triki-Ellouz, Y. and Nasri, M. 2007. Biochemical and Functional Studies Center. University of Basrah. Alrashad Press, Baghdad, 546p.

Taheri, A., Anvar, S.A.A., Ahari, H. and Fogliano, V. 2013. Comparison the functional properties of protein Hydrolysates from poultry byproducts and rainbow trout) *Onchorhynchus mykiss* viscera. Iranian Journal of Fisheries Science, 12: 154-169. http://hdl.handle.net/1834/37351.

Tounkara, F., Sodio, B., Amza, T., Le, G.W. and Shi, Y.H. 2013. Antioxidant effect and water holding capacity of roselle *Hibiscus sabdariffa* L. seed protein hydrolysates. Advance

Journal of Food Science and Technology, 5(6): 752-757. https://maxwellsci.com/msproof.php?doi=ajfst.5.3159.

Wilding, P., Lillford, P.J. and Regenstein, J.M. 1984. Functional properties of proteins in foods. J. Chem. Technol. Biotechnol., 34B: 182-189.https://doi.org/10.1002/jctb.280340307

Xue, M. and Cui, Y.B. 2001. Effect of several feeding stimulants on diet preference by juvenile gibel carp *Carassius auratus* gibelio, fed diets with or without partial replacement of fishmeal by meat and bone meal. Aquaculture, 198: 281-292.https://doi.org/10.1016/S0044-8486(00)00602-5