Food Research 8 (Suppl. 2) : 139 - 147 (2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Optimization of cobia fish (*Rachycentron canadum*) gelatin extraction with response surface methodology

[1,2,4]Anandito, R.B.K., [3]Purwanto, E., [4,*]Praseptiangga, D. and [4]Zaman, M.Z.

[1]*Doctoral Program of Agricultural Science, Faculty of Agriculture, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia*
[2]*Department of Agricultural Product Technology, Vocational School, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia*
[3]*Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia*
[4]*Department of Food Science and Technology, Faculty of Agriculture, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia*

Article history:
Received: 5 March 2023
Received in revised form: 27 September 2023
Accepted: 20 January 2024
Available Online: 16 April 2024

Keywords:
Gelatin,
Cobia fish,
Optimization,
Response surface methodology

DOI:
https://doi.org/10.26656/fr.2017.8(S2).98

Abstract

Cobia fish or often called sea cork fish, is a fish that has thick meat which is nutritionally equivalent to salmon meat. Cobia fish is usually sold in the form of fish fillets and the bones becomes solid waste, which has the potential to be a raw material for fish gelatin. Gelatin is a bone and skin collagen hydrolysis product that can be used for food and non-food industries. The quality of fish gelatin is influenced by the extraction process, such as acid concentration, temperature, and extraction time. This study aims to determine the effect of hydrochloric acid concentration, temperature, extraction time, and the optimal process of making cobia fish bone gelatin using the response surface methodology with the Box Behnken model. The data was processed using Design Expert 11 software. The model suggested by the software was the cubic model. The optimum extraction conditions obtained were using a concentration of 5.53% hydrochloric acid, a temperature of 90℃, and a stirring time of 6.42 mins would produce a yield of 12.38% and gel strength of 229.14 bloom. The verification results by extracting cobia fish gelatin with optimum conditions obtained a yield of 11.22% and gel strength of 227.60 bloom. The proximate analysis of cobia fish gelatin extracted under optimum conditions was found to contain 6.43% water content, 8.27% ash content, 4.65% fat content, 68.47% protein content, and 12.18% carbohydrate by different. Cobia fish gelatin microstructure is in the form of thick strands with a soft sponge.

1. Introduction

Cobia fish (*Rachycentron canadum*) is a marine fish belonging to large carnivores in tropical and subtropical waters worldwide except in the East Pacific. Cobia fish are usually found in shallow waters on the coast and bay waters. Most eggs and larvae are found offshore (Zulhusni *et al*., 2017). The growth of cobia fish is fast, efficient, and has high product quality, so it is easy to cultivate (Trushenski *et al*., 2011). In Indonesia, cobia fish have started to be cultivated. The Ministry of Marine Affairs and the Fisheries Republic of Indonesia hope cobia fish can become a new Indonesian aquaculture commodity (Yustiati *et al*., 2021). The growth of cobia fish can reach six to eight kilograms in one year, making it suitable to meet market needs. All parts of the cobia fish body can be utilized to have high economic value (Wu *et al*., 2020).

One part of the cobia fish body that can be used is the bone. Fish bones are one of the waste products from the fishing industry that have not been used correctly. Waste in the form of fish bones ranges from 9-15%, depending on the type of fish. This fishbone waste has not been utilized optimally (Coppola *et al*., 2021). Fish bones contain minerals that can be utilized, including calcium, magnesium, sodium, and potassium (Kandyliari *et al*., 2020). These minerals, especially calcium, are utilized by the body as a substitute for calcium from milk or other supplements (Rosidi *et al*., 2021). Another use of fish bones is made into gelatin. Gelatin can be obtained from the partial hydrolysis of collagen by extraction in hot water combined with acid or alkaline treatment (Alipal *et al*., 2021).

*Corresponding author.
Email: *dpraseptiangga@staff.uns.ac.id*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

Many kinds of research on gelatin extraction have been carried out. Nuryanto *et al*. (2018) extracted gelatin from catfish bones using a combination of acid and base at 70℃ for 5 hrs, resulting in a gelatin yield of 7%. Arshad *et al*. (2021) produced the best sardine bone gelatin extracted using hydrochloric acid at 67℃ for 14 hrs. While Atma and Ramdhani (2017) extracted catfish bone gelatin, the best extraction temperature was 75℃ for 5 hrs resulting in a yield of 6.12%. Based on the research that has been done, we need an optimization method for extracting fishbone gelatin so that optimum results are obtained.

Response surface methodology (RSM) is an optimization method with an experimental design that uses several variables that affect a system (Suliman, 2017). Optimization of gelatin extraction using RSM has been carried out by Arpi *et al*. (2016), who extracted gelatin from Triggerfish skin with optimum extraction conditions, there is an acid concentration of 0.95 N, the base concentration of 0.55 N, and an extraction temperature of 90℃. Jakhar *et al*. (2014) optimized the extraction of black-spotted croaker skin gelatin with optimum conditions of 0.2% NaOH concentration, 46.68 mins of immersion time, 56.23℃ of temperature, and 15.21 hrs of extraction. In contrast, Mardiyah *et al*. (2019) extracted gelatin from pink perch fish heads with an optimum extraction temperature of 74.40℃ for 5.42 hrs. Until now, there has been no optimization of the extraction conditions for gelatin from cobia fish bones, so this is the first time this research has been carried out. This study aimed to determine the optimum extraction conditions for cobia fish bone gelatin using the response surface methodology.

2. Materials and methods

2.1 Materials

The main ingredient in this study was Cobia fish bone obtained from Pasar Gang Baru, Semarang City. Chemicals such as HCl from (Merck, Germany).

2.2 Cobia fish bone gelatin extraction

The extraction method was performed according to Mardiyah *et al*. (2019), with modifications. Fish bones are washed with running water, then boiled in boiling water (degreasing) to remove fat, dirt, and remaining meat that is still attached to the bones. Degreasing was carried out at 80℃ for 30 mins. The bone is dried, then cut into pieces with a size of 1.5-2 cm. The bones were then immersed in HCl solution at a concentration of 2%, 4% and 6%, with a ratio of fish bone and HCl solution (1:4) for 48 hrs to form ossein. The bones were washed with running water to remove the HCl solution attached to the ossein. Ossein was extracted in a water bath using distilled water with the ratio between ossein and distilled water (1:3) at 70℃, 80℃ and 90℃ for 5, 6 and 7 hrs. The gelatin solution formed is filtered to remove other substances that can reduce the purity of the gelatin. After filtering, the gelatin solution was placed in an aluminum pan, then dried in an oven at 55℃ for 48 hrs to form gelatin sheets. The gelatin sheets were crushed using a blender to obtain gelatin powder.

2.3 Yield

The yield of cobia fish gelatin was calculated based on Ratnasari *et al*. (2013) by dividing the weight of the gelatin obtained by the weight of the extracted cobia fish bones multiplied by 100%.

2.4 Gel strength

Gel strength analysis was carried out based on Zhang *et al*. (2021) using texture testing using a Texture analyzer (TA TX Analyzer) (Brookfield, USA) by preparing a gelatin solution in 6.7% (w/v) distilled water and stored at 10℃ for 18 hrs and then analyzed.

2.5 Proximate analysis

Proximate analysis was carried out based on the AOAC (2005). The moisture content was measured by the thermogravimetric method, the ash content by the dry method, the fat content by the Soxhlet method, the protein content by the Kjeldahl method, and the carbohydrate content based on the difference of 100 minus the amount of water, ash, fat content, and protein (by different).

2.6 Amino acid profile

Amino acid analysis was carried out based on Rahayu *et al*. (2017) using the LCMS MassLynx V4.1 SCN940 (The Waters Xevo TDQ, Gen Tech Scientific, USA). The analysis was done by dissolving 1 g of gelatin into 20 mL of 6 N HCl and then hydrolyzing it in an autoclave at 110℃ for 12 hrs. The solution was then neutralized with 6N NaOH to a volume of 100 mL. The solution was diluted with distilled water as much as ten times the dilution. The solution was then filtered through a 0.22 µM filter. The 5 µM solution was then injected into LCMSMS using AcquityUPLC column C18, 1.7µm, 2.1×50mm with mobile phase A: 0.1% Pentadecafluorooctanoic Acid (PDFOA) 99.5%: 0.5% Water/CH_3CN with 0, 1% Formic acid and B: 0.1% PDFOA, 10%: 90% Water/CH_3CN with 0.1% Formic acid with flow 0.6 mL/min.

2.7 Scanning electron microscopy

Morphological analysis was carried out based on Subara and Jaswir (2021) using scanning electron

https://doi.org/10.26656/fr.2017.8(S2).98

microscopy (JSM-6510LA, Jeol Ltd, Japan) with a voltage of 20 kV and a gold coating.

2.8 Statistical analysis

This research was carried out in triplicate. Optimization of cobia fish bone gelatin extraction using response surface methodology (RSM) with Box–Behnken design using three variables; HCl concentration, extraction temperature, and extraction time. Also, its effect on yield and gel strength with treatment combinations is presented in Table 1. Data were analyzed using Design Expert 11.1.2.0.

3. Results and discussion

3.1 Yield

The model selection is based on Table 2, the summary statistics of RSM suggest a quadratic model with a standard deviation value of 0.99, which is a smaller value than the other models. The quadratic model has an Adjusted R^2 value of 0.92 and a predicted R^2 of 0.71. This data shows that the variable HCl concentration, temperature, and time affect the gelatin yield response by 92%, and the remaining 8% is caused by other factors that are not used as variables studied. Chen *et al*. (2009) stated that a good model has an R^2 value of more than 0.80. Model selection is also focused on the highest PRESS value, which is 52.00, so this quadratic model is suggested in the summary analysis of the model.

Table 3 shows the ANOVA test used to determine the response to variables in the gelatin-making process. The F-value in the model is 20.26, and the P-value is 0.0020; it is said that the model is significant because it has a p-value of less than 0.05. In the lack of fit, the p-value is 1.03 while the p-value is 0.52, indicating that the value is insignificant. Nasir *et al*. (2016), an insignificant lack of fit is a requirement for a good model because it shows the suitability of the response data with the model. The mathematical model for the yield response is Yield = 4.19 + 3.76*A + 1.68*B + 0.77*C + 0.28*AB + 0.40*AC + 0.63*BC + 0 ,59*A2 +1.90*B2 + 2.62*C2, where A is the concentration of HCl, B is temperature and C is time.

Figure 1 is a two-dimensional graph of the yield response. This graph analyzes the interaction between temperature, HCl concentration and stirring time of fishbone gelatin extraction on the yield response. While in Figure 2 is a three-dimensional graph of a quadratic model in the form of a parabola (curved). The blue color in the graph is the minimum response value, while the orange color indicates the maximum response value. It proves that the higher the concentration of HCl, the temperature, and the time used at the extraction time, the more yield will be produced. The three-dimensional

Table 1. Box-Behnken design three variables on yield and gel strength result.

Run	HCL concentration (%)	Extraction temperature (°C)	Extraction time (hrs)	Yield (%)	Gel strength (bloom)
1	2	70	6	1.15	100.56
2	6	70	6	9.17	217.42
3	2	90	6	3.47	111.34
4	6	90	6	12.63	225.60
5	2	80	5	3.88	83.35
6	6	80	5	9.50	129.20
7	2	80	7	4.33	120.60
8	6	80	7	11.57	151.77
9	4	70	5	6.47	105.65
10	4	90	5	9.02	140.55
11	4	70	7	7.14	127.60
12	4	90	7	12.22	195.98
13	4	80	6	5.33	251.17
14	4	80	6	3.69	291.20
15	4	80	6	3.57	280.61

Table 2. Model summary statistics.

Parameters	Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Yield	Linear	1.99	0.7630	0.6983	0.6192	70.09	
	2FI	2.27	0.7770	0.6097	0.3456	120.45	
	Quadratic	0.9912	0.9733	0.9253	0.7175	52.00	Suggested
	Cubic	0.9833	0.9895	0.9265		*	Aliased
Gel strength	Linear	0.3653	0.0745	0.0351	-0.1121	0.3653	
	2FI	0.9965	0.0506	-0.3179	-0.8601	0.9965	
	Quadratic	0.0055	0.2587	0.7998	0.0339	0.0055	Suggested
	Cubic	0.2587		0.9095		0.2587	Aliased

https://doi.org/10.26656/fr.2017.8(S2).98

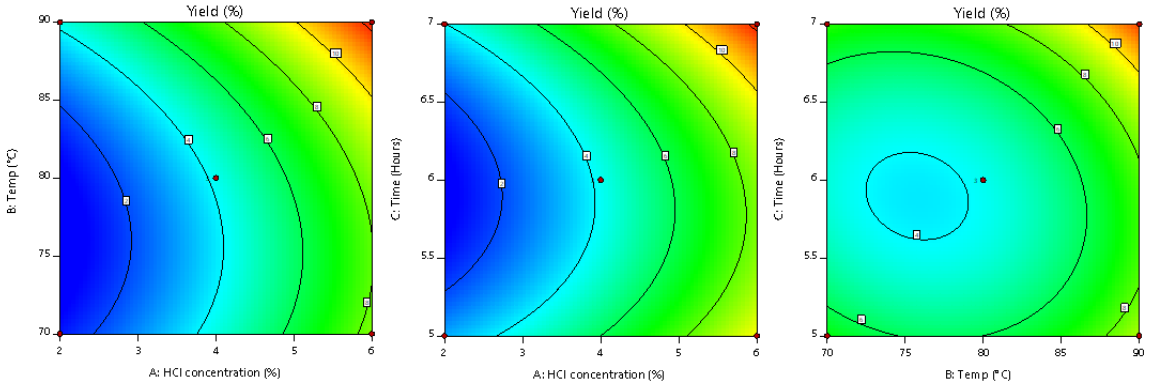

Figure 1. 2D graph response of yield.

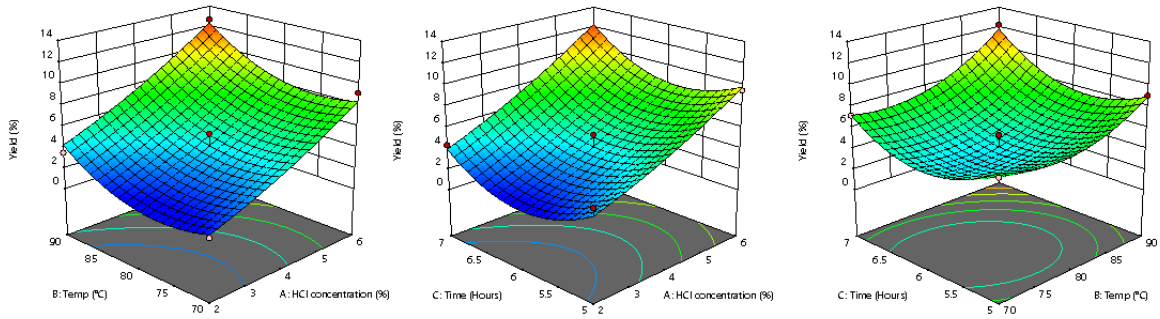

Figure 2. 3D graph response of yield.

graph shows that the highest yield was obtained from treatment using 6% HCl concentration with a temperature of 90°C and stirring time for 6 mins to produce a yield of 12.62%. The results of this study were higher than the study of Afrian and Suprayitno (2019); the highest yield of catfish bone gelatin was 11.06%. The higher the temperature and extraction time, the higher the yield value. The number of H+ ions that hydrolyze collagen is more significant, so the longer the extraction time causes more collagen to break down into gelatin.

3.2 Gel strength

The recommended model of the system is the quadratic model, which is in Table 2. This quadratic model has the slightest standard deviation than the other models, namely 0.0055, with an R^2 value of 0.25. An adjusted R^2 of 0.79 means a correlation, and the actual data for the gel strength response included in this linear model is 79%. The predicted R^2 is 0.03. Maran *et al.* (2013) state that the model selection can be seen from the model with a maximum value of R^2, adjusted R^2, and predicted R^2, as well as a minimum standard deviation and PRESS value (0.05). The quadratic model in this study is the most suitable model for extracting gelatin from cobia fish bones. The accuracy of using this model can also be seen from the ANOVA analysis.

The ANOVA analysis in Table 3 shows that the model chosen for the gel strength response is quadratic. The P-value of 0.021 can be interpreted as a significant model with a value less than 0.05. So, it can be concluded that the existing variables significantly affect the gel strength response. The lack of fit value of 0.25 also shows that this value is insignificant, with a p-value of more than 0.05. The ANOVA table also has an adeq precision value of 8.43, indicating good results. Behera *et al*. (2018) stated that the signal-to-noise ratio could be measured by the value of adequacy precision, consisting

Table 3. ANOVA results of yield and gel strength of cobia fish gelatin.

Parameters	Source	Sum of squares	Df	Mean square	F-value	P-value	
Yield	Model	179.16	9	19.91	20.26	0.0020	Significant
	A-HCl concentration	112.82	1	112.82	114.84	0.0001	
	B-Extraction temp.	22.50	1	22.50	22.90	0.0049	
	C-Extraction time	5.11	1	5.11	5.21	0.0714	
	Residual	4.91	5	0.98			
	Lack of Fit	2.98	3	0.99	1.03	0.5279	not significant
	Pure Error	1.93	2	0.97			
Gel strength	Model	61765.77	9	6862.86	7.21	0.0212	Significant
	A-HCl concentration	11868.78	1	11868.78	12.48	0.0167	
	B-Extraction temp.	1867.83	1	1867.83	1.96	0.2200	
	C-Extraction time	2352.98	1	2352.98	2.47	0.1766	
	Residual	4756.05	5	951.21			not significant
	Lack of Fit	3895.63	3	1298.54	3.02	0.2587	
	Pure Error	860.42	2	430.21			

https://doi.org/10.26656/fr.2017.8(S2).98

of the predicted value at the design point and the average prediction error. The relationship between the variables and the response is said to be good if it has an adeq precision of more than 4. The mathematical equation for the gel strength response is gel strength = 274.33 + 38.52*A + 15.28*B + 17.15*C – 0.65 *AB – 3.67*AC + 8.37*BC – 65.91*A2 – 44.69*B2 – 87.19*C2, where A is concentration, B is temperature and C is time.

Two-dimensional graphs (Figure 3) and three-dimensional (Figure 4) on the gel strength response show that the higher the HCl concentration, temperature, and stirring time at the extraction time, the greater the gel strength value. The highest gel strength value was treated with 4% HCl concentration, a temperature of 80°C with a stirring time of 6 mins resulting in a gel strength of 291.2 blooms. Based on research by Ismail and Abdullah (2016), the highest gel strength in tilapia skin gelatin was 180.76 with a concentration of 0.05 M HCl. The difference in the HCl concentration will affect the gel's strength. It can happen because adding HCl will affect the pH value and isoelectric point. When the pH is adjusted closer to the isoelectric point, the gel formed will be firmer and more robust since the gelatin polymers are close to each other. These results indicate that each acid concentration during pre-treatment can obtain different gel strengths, which can be suitable for different applications.

3.3 Optimum conditions for extraction of cobia fish gelatin

This optimization process aims to minimize operational costs by producing high-quality products. The optimization limits of HCl concentration, temperature, and time can be seen in Table 4. The HCl concentration variables were optimized in the range of 2-6%, the temperature ranged from 70-90%, and the stirring time was 5-7 mins with importance 3. The concentration of HCl, temperature, and stirring time will affect the quality of the gelatin. The yield response and gel strength were chosen with the maximum target because the goal was to obtain the product with the highest yield from the various treatments tested. Based on the criteria from Table 4, the optimum result predicted by the design expert was fish bone gelatin with extraction treatment with an HCl concentration of 5.53%, a temperature of 90°C, and a stirring time of 6.42 mins would produce a yield of 12.38% and gel strength of 229.14 bloom with the desirability value of the solution of 0.82.

3.4 Characteristics of cobia fish gelatin extracted at optimum conditions

Based on the optimum conditions obtained from the analysis with RSM, then verification was carried out by extracting cobia fish gelatin. The cobia fish gelatin

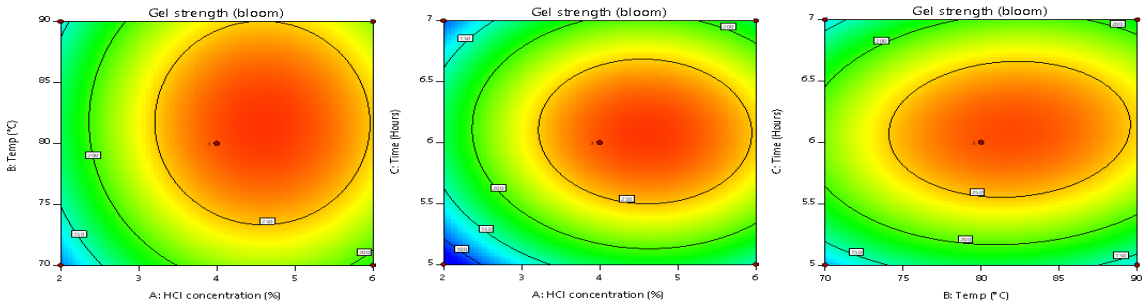

Figure 3. 2D graph response of gel strength.

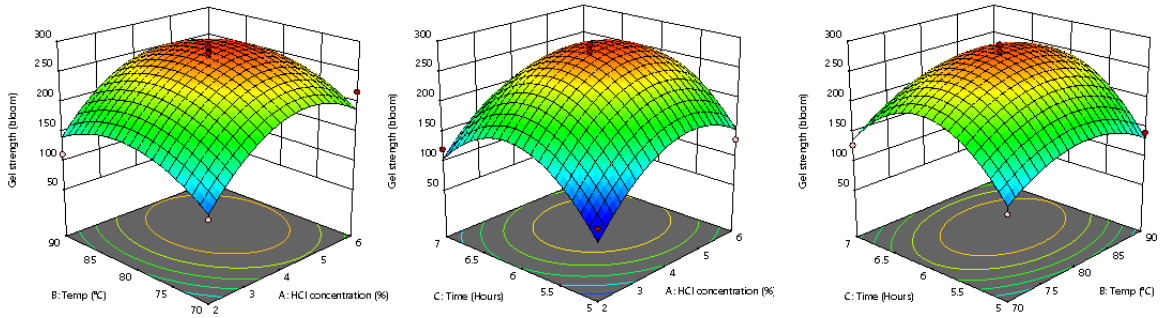

Figure 4. 3D graph response of gel strength.

Table 4. Optimisation response.

Name	Goal	Lower limit	Upper limit	Importance
HCl concentration	Is in range	2	6	3
Extraction temperature	Is in range	70	90	3
Extraction time	Is in range	5	7	3
Yield	maximize	1.15	12.63	3
Gel strength	maximize	83.35	291.20	3

https://doi.org/10.26656/fr.2017.8(S2).98

Table 5. Characteristics of cob

obtained was then characterized by chemical composition (yield, gel strength, amino acids, and functional groups), morphology and particle size. The chemical composition of cobia fish gelatin is shown in Table 5. Extraction of cobia fish gelatin with optimum conditions obtained a yield of 11.22% and a gel strength of 227.60 bloom. The yield and gel strength analysis results are lower than the predictions of expert design analysis results, namely yield of 12.38% and gel strength of 229.14 blooms.

Table 5. Characteristics of cobia fish gelatin extracted at optimum conditions based on RSM analysis.

Parameter	Value
Yield (%)	11.22
Gel strength (bloom)	227.60
Water content (%)	6.43
Ash content (%)	8.27
Fat content (%)	4.65
Protein content (%)	68.47
Carbohydrate content by different (%)	12.18

The proximate analysis results of cobia fish gelatin extracted under optimum conditions were found to contain 6.43% water content, 8.27% ash content, 4.65% fat content, 68.47% protein content, and 12.18% carbohydrate by different. Based on the results of the proximate analysis obtained, it is known that cobia fish gelatin is high in protein and low in fat. The proximate yield of cobia fish gelatin is lower than that of red snapper bone gelatin and grouper fish, with a protein content of 78.56% and 82.36%, respectively (Atma, 2017). The collagen content influenced these different results in each fishbone because gelatin was extracted from bone collagen (Mahmood *et al*., 2016). The high protein content in cobia fish gelatin affects the amino acid content.

The amino acids of cobia fish gelatin are presented in Table 6. The dominant amino acids found in cobia fish gelatin are arginine, glycine, lysine, and tryptophan, while other amino acids detected are glutamic acid, leucine, phenylalanine, valine, serine, and methionine. The amino acids such as aspartic acid, isoleucine, histidine, threonine, proline, tyrosine, and alanine were also detected in small amounts. Different results were shown by Derkach *et al*. (2020), where the dominant amino acid content in cod fish gelatin was glycine, alanine, proline, and arginine. Meanwhile, Siregar and Suprayitno (2019) stated that grouper gelatin's dominant amino acid content is glycine, arginine, alanine, and proline.

The amino acid content of fish gelatin depends on the fish used. In addition, the amino acid content of gelatin is influenced by the extraction conditions that affect the protein fragmentation process, where gelatin is a breakdown product of fibrillar protein (collagen) in bone or skin in the presence of an acid, base, or enzyme treatment. Collagen is a fibrillar protein composed of tropocollagen consisting of three polypeptide bonds that form a triple helix. These triple helical bonds will break during denaturation at high temperatures and produce gelatin. The broken peptide bonds during the high-temperature denaturation process will form protein fragments that are affected by the length of heat treatment (Atma, 2017; Derkach *et al*., 2019). Cobia fish are fish that live in cold water (Wu *et al*., 2020). Gelatin extracted from cold water fish contains the amino acids glycine, serine, aspartic acid, threonine, and methionine, while the amino acids proline and hydroxyproline are in low amounts. The low proline and hydroxyproline affect the rheological properties. Thus, the application of gelatin extracted from cold water fish in food products is limited (Alfaro *et al*., 2014). Gelatin from cold-water fish is very suitable for edible films (Nitsuwat *et al*., 2021).

The microstructure of cobia fish gelatin is shown in Figure 5. Cobia fish gelatin forms thick strands with a

Table 6. Amino acid profile of cobia fish gelatin extracted at optimum conditions based on RSM analysis.

No	Amino acids	Value
Non-essential amino acids		
1	L-Alanine	0.04
2	L-Arginine	12.01
3	L-Proline	0.27
4	L-Aspartic acid	0.86
5	L-Glutamate acid	1.51
6	L-Cysteine	<0.01
7	L-Glycine	3.93
8	L-Tyrosine	0.10
	Total Non-essential amino acids	18.72
Essential amino acids		
1	L-Histidine	0.57
2	L-Isoleucine	0.85
3	L-Leucine	1.19
4	L-Lysine	3.34
5	L-Phenylalanine	1.35
6	L-Threonine	0.64
7	L-Tryptophan	3.80
8	L-Valine	1.30
9	L-Serine	1.52
10	L-Methionine	1.28
Total Essential amino acids		15.84

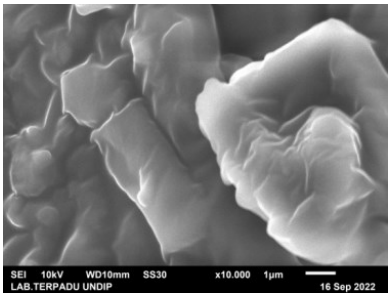

Figure 5. Morphology of cobia fish gelatin extracted at optimum conditions based on RSM analysis.

https://doi.org/10.26656/fr.2017.8(S2).98

soft sponge. The results in this study follow that shown by Arshad *et al*. (2021), where the gelatin microstructure of Sardinella fish bones is in the form of a sponge shape. The formation of microstructures is related to gel strength which is influenced by the combination of protein molecules and the presence of α, β, and γ bonds in the gel matrix (Ratnasari and Firlianty, 2016; Arshad *et al*., 2021).

4. Conclusion

The highest yield was obtained from treatment using 6% HCl concentration with a temperature of 90°C and stirring time for 6 mins to produce a yield of 12.62%. The highest gel strength value was treated with 4% HCl concentration, a temperature of 80°C with a stirring time of 6 mins resulting in a gel strength of 291.2 blooms. Cobia fish gelatin extracted optimum conditions with an HCl concentration of 5.53%, a temperature of 90°C, and a stirring time of 6.42 mins would produce a yield of 12.38% and gel strength of 229.14 bloom with a desirability value of the solution of 0.82. The verification results by extracting cobia fish gelatin with optimum conditions obtained a yield of 11.22% and gel strength of 227.60 bloom. The proximate analysis of cobia fish gelatin extracted under optimum conditions was found to contain 6.43% water content, 8.27% ash content, 4.65% fat content, 68.47% protein content, and 12.18% carbohydrate by different. Cobia fish gelatin microstructure is in the form of thick strands with a soft sponge.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This research was funded by Hibah Penelitian Disertasi Doktor Universitas Sebelas Maret 2021.

References

Afrian, D. and Suprayitno, E. (2019). The effect of a long time of NAOH seeding in the loss process fat to the quality of gelatin tiger grouper fish bone (*Epinephelus fuscoguttatus*). *IOSR Journal of Agriculture and Veterinary Science*, 12(1), 62-66. DOI: 10.9790/2380-1205016266

Alfaro, A.D.T., Balbinot, E., Weber, C.I., Tonial, I.B. and Machado-Lunkes, A. (2014). Fish gelatin: characteristics, functional properties, applications, and future potentials. *Food Engineering Reviews*, 7 (1), 33-44. https://doi.org/10.1007/s12393-014-9096-5

Alipal, J., Pu'ad, N.M., Lee, T.C., Nayan, N.H.M., Sahari, N., Basri, H., Idris, I. and Abdullah, H.Z. (2021). A review of gelatin: Properties, sources, process, applications, and commercialisation. *Materials Today: Proceedings*, 42(1), 240-250. https://doi.org/10.1016/j.matpr.2020.12.922

Association of the Analytical Chemists (AOAC). (2005). Official Methods of Analysis. Washington DC, USA: AOAC International.

Arpi, N., Fahrizal, Satriana and Edward, S. (2016). Extraction and properties of gelatin from spotted oceanic triggerfish (*Canthidermis maculata*) skin and bone. *International Journal on Advanced Science, Engineering and Information Technology*, 6 (5), 561-567. https://doi.org/10.18517/ijaseit.6.5.797

Arshad, N.M., Ghaffar, M.A. and Mohtar, N.F. (2021). Optimization of the extraction procedures and the characterization of fish gelatin from fringescale sardinella (*Sardinella fimbriata*) bones. *Aquaculture, Aquarium, Conservation and Legislation*, 14(2), 672 -682.

Atma, Y. (2017). Amino acid and proximate composition of fish bone gelatin from different warm-water species: A comparative study. *IOP Conference Series: Earth and Environmental Science,* 58(1), 012008. https://doi.org/10.1088/1755-1315/58/1/012008

Atma, Y. and Ramdhani, H. (2017). Gelatin extraction from the indigenous Pangasius catfish bone using pineapple liquid waste. *Indonesian Journal of Biotechnology*, 22(2), 86-91. https:// doi.org/10.22146/ijbiotech.32472

Behera, S.K., Meena, H., Chakraborty, S. and Meikap, B.C. (2018). Application of response surface methodology (RSM) for optimization of leaching parameters for ash reduction from low-grade coal. *International Journal of Mining Science and Technology*, 28(4), 621-629. https://doi.org/10.1016/ j.ijmst.2018.04.014

Chen, H.H., Lin, C.H. and Kang, H.Y. (2009). Maturation effects in fish gelatin and HPMC composite gels. *Food Hydrocolloids*, 23(7), 1756-1761. https://doi.org/10.1016/j.foodhyd.2009.03.004

Coppola, D., Lauritano, C., Esposito, F.P., Riccio, G., Rizzo, C. and Pascale, D. (2021). Fish waste: from problem to valuable resource. *Marine Drugs*, 19(2), 116. https://doi.org/10.3390/md19020116

Derkach, S.R., Kuchina, Y.A., Baryshnikov, A.V., Kolotova, D.S. and Vorońko, N.G. (2019). Tailoring cod gelatin structure and physical properties with acid and alkaline extraction. *Polymers*, 11(10), 1724. https://doi.org/10.3390/polym11101724

Derkach, S.R., Vorońko, N.G., Kuchina, Y.A. and Kolotova, D.S. (2020). Modified fish gelatin as an alternative to mammalian gelatin in modern food technologies. *Polymers*, 12(12), 3051. https://doi.org/10.3390/polym12123051

Ismail, N. and Abdullah, H.Z. (2016). Effects of hydrochloric acid concentration on the properties of black tilapia fish skin gelatin. *Materials Science Forum*, 840, 165-169. https://doi.org/10.4028/www.scientific.net/MSF.840.165

Jakhar, J.K., Basu, S., Sasidharan, S., Chouksey, M.K. and Gudipati, V. (2014). Optimization of process parameters for gelatin extraction from the skin of Blackspotted croaker using response surface methodology. *Journal of Food Science and Technology*, 51(11), 3235-3243. https://doi.org/10.1007/s13197-012-0883-9

Kandyliari, A., Mallouchos, A., Papandroulakis, N., Golla, J.P., Lam, T.T., Sakellari, A., Karavoltsos, S., Vasiliou, V. and Kapsokefalou, M. (2020). Nutrient composition and fatty acid and protein profiles of selected fish by-products. *Foods*, 9(2), 190. https://doi.org/10.3390/foods9020190

Mahmood, K., Muhammad, L., Ariffin, F., Kamilah, H., Razak, A. and Sulaiman, S. (2016). Review of fish gelatin extraction, properties and packaging applications. *Food Science and Quality Management*, 56, 47-59.

Maran, J.P., Manikandan, S., Thirugnanasambandham, K., Nivetha, C.V. and Dinesh, R. (2013). Box-Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk polysaccharide. *Carbohydrate Polymers*, 92(1), 604-611. https://doi.org/10.1016/j.carbpol.2012.09.020

Mardiyah, U., Widjanarko, S.B. and Fibrianto, K. (2019). Optimization of time and temperature gelatin extraction from pink perch (*Nemipterus bathybius*) head using response surface methodology (RSM). *The Journal of Experimental Life Science*, 9(2), 97-104. https://doi.org/10.21776/ub.jels.2019.009.02.06

Nasir, S.M., Ismail, K.A. and Shayfull, Z. (2016). Application of RSM to optimize moulding conditions for minimizing shrinkage in thermoplastic processing. *Key Engineering Materials,* 700, 12-21. https://doi.org/10.4028/www.scientific.net/KEM.700.12

Nitsuwat, S., Zhang, P., Ng, K. and Fang, Z. (2021). Fish gelatin as an alternative to mammalian gelatin for food industry: A meta-analysis. *LWT - Food Science and Technology*, 141, 110899. https://doi.org/10.1016/j.lwt.2021.110899

Nuryanto, R., Trisunaryanti, W. and Falah, I.I. (2018). Extraction of gelatin from catfish bone using NaOH and its utilization as a template on mesoporous silica alumina. *IOP Conference Series: Materials Science and Engineering,* 349, 012051. https://doi.org/10.1088/1757-899X/349/1/012051

Rahayu, W.S., Rohman, A. and Martono, S. (2017). Identification of dog gelatin based on amino acid profiles and chemometrics. *Pharmacy*, 14(2), 212-219. https://doi.org/10.30595/pharmacy.v14i2.2021

Ratnasari, I. and Firlianty. (2016). Physico-chemical characterization and skin gelatin rheology of four freshwater fish as alternative gelatin source. *Aquaculture, Aquarium, Conservation and Legislation*, 9(6), 1196-1207.

Ratnasari, I., Yuwono, S.S., Nusyam, H. and Widjanarko, S.B. (2013). Extraction and characterization of gelatin from different freshwater fishes as alternative sources of gelatin. *International Food Research Journal*, 20(6), 3085-3091.

Rosidi, W.N.T.M., Arshad, N.M. and Mohtar, N.F. (2021). Characterization of *Sardinella fimbriata* and *Clarias gariepinus* bones. *Biodiversitas Journal of Biological Diversity*, 22(4), 1621-1626. https://doi.org/10.13057/biodiv/d220405

Siregar, G.R.M. and Suprayitno, E. (2019). Amino acid composition of gelatin from *Ephinephelus* sp. *IOSR Journal of Agriculture and Veterinary Science*, 12 (4), 51-54.

Subara, D. and Jaswir, I. (2021). The effect of processing parameters on the properties of fish gelatin hydrolysate nanoparticle. *Journal of Science and Applicative Technology*, 5(1), 17-24. https://doi.org/10.35472/jsat.v5i1.417

Suliman, R. (2017). Response surface methodology and its application in optimizing the efficiency of organic solar cells. South Dakota, USA: South Dakota State University. Thesis.

Trushenski, J., Schwarz, M., Lewis, H., Laporte, J., Delbos, B., Takeuchi, R. and Sampaio, L.A. (2011). Effect of replacing dietary fish oil with soybean oil on production performance and fillet lipid and fatty acid composition of juvenile cobia *Rachycentron canadum*. *Aquaculture Nutrition*, 17(2), 437-447. https://doi.org/10.1111/j.1365-2095.2010.00779.x

Wu, Y.L., Lee, M.A., Chen, L.C., Chan, J.W. and Lan, K.W. (2020). Evaluating a suitable aquaculture site selection model for Cobia (*Rachycentron canadum*) during extreme events in the inner bay of the Penghu Islands, Taiwan. *Remote Sensing*, 12(17), 2689. https://doi.org/10.3390/rs12172689

Yustiati, A., Bangkit, I. and Pratiwi, D.Y. (2021). Pengembangan Komoditas Perikanan. Retrieved

from https://www.researchgate.net/publication/353917005_Pengembangan_Komoditas_Perikanan [In Bahasa Indonesia].

Zhang, W., Chen, C., Huang, Z. and Wang, P. (2021). Characterizations of gelatin from the skin of american bullfrog (*Rana catesbeiana*) as affected by extraction temperature. *Sustainability*, 13(8), 4390. https://doi.org/10.3390/su13084390

Zulhusni, A., Mazlan, A.G., Zaidi, C.C. and Samat, A. (2017). Diet composition of cobia fish, *Rachycentron canadum* (Linnaeus, 1766) from northeastern waters of Peninsular Malaysia. *Malaysian Applied Biology Journal*, 46(1), 219-224.

https://doi.org/10.26656/fr.2017.8(S2).98