Isolation of fish skin and bone gelatin from tilapia (*Oreochromis niloticus*): Response surface approach

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Abstract. In this study, gelatin from fish collagen, as one of halal sources, was extracted from tilapia (Oreochromis niloticus) skin and bone, by using Response Surface Methodology to optimize gelatin extraction conditions. Concentrations of alkaline NaOH and acid HCl, in the pretreatment process, and temperatures in extraction process were chosen as independent variables, while dependent variables were yield, gel strength, and emulsion activity index (EAI). The result of investigation showed that lower NaOH pretreatment concentrations provided proper pH extraction conditions which combine with higher extraction temperatures resulted in high gelatin yield. However, gelatin emulsion activity index increased proportionally to the decreased in NaOH concentrations and extraction temperatures. No significant effect of the three independent variables on the gelatin gel strength. RSM optimization process resulted in optimum gelatin extraction process conditions using alkaline NaOH concentration of 0.77 N, acid HCl of 0.59 N, and extraction temperature of 66.80 °C. The optimal solution formula had optimization targets of 94.38%.

1. Introduction

Gelatin is a translucent, colorless, brittle (when dry), flavorless, edible, multifunctional natural macromolecule protein polymer which is extracted by thermal hydrolysis of collagen tissue found in bones, skin, and connective tissue of various animal and fish [1][2][3]. Industries utilize gelatin extensively as a gelling agent, emulsifier, stabilizer, adhesives, viscosity agent and binder agent. Worldwide gelatin consumption is to reach 395.84 thousand metric tons by the year 2017. The vast majority of commercial gelatin is derived from porcine skin (46%), bovine hide (29.4%), bones (23.1%) and other sources (1.5%) [4]. Demand for alternative gelatin source is increasing due to religious reasons. Porcine gelatin cannot be used in kosher (Jewish) and halal (Muslims) foods, while Hindus do not consume bovine gelatin. The use of fish byproducts for alternative gelatin production is growing. Fish byproducts such as skin, bones, fins and scales account for ± 21% [5], even ± 50% for certain fish. Tilapia (*Oreochromis niloticus*) consumption in Indonesia is rapidly increasing and utilization of skin and bone are expected to reduce waste and increase the value of the waste. Fish gelatin properties influenced by many factors, such as species, water temperature fish habitats, and the

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gelatin extraction process, which may depend on pH, temperature, and time during pretreatment and extraction process [6]. The objectives of this research were to study the effects of pretreatments using alkaline and acid on gelatin extraction, to characterize tilapia gelatin, and to determine optimum conditions for gelatin extraction from tilapia fish skin and bone.

2. Method

In order to obtain the optimum conditions of gelatin extraction, a Response Surface Methodology (RSM) approach was used. RSM using design expert software (9.0.5 version) was used to obtain several optimum gelatin extraction conditions. Mathematical techniques combine with statistical techniques used in the RSM method to create and analyze Y response (e.g. yield, gel strength, etc.) which are influenced by independent variables or X factor. Three independent variables (X factors) used in this study, i.e. X_1= alkaline (NaOH) concentration, and X_2= acid (HCl) concentration in the pretreatment process, and X_3= temperature of the extraction process which were tested at 5 different levels using 3 factors and 5 levels central composite rotatable design (Table 1). Levels of independent variables were obtained after the central composite rotatable design was run (Table 2). Response factors (=Y response) observed were yield [7], emulsion activity index [8], and gel strength [9].

Table 1. The independent variables and the levels of independent variables in 3 factors and 5 levels central composite rotatable design.

Independent Variables	Symbol	Code Level				
		-α	**-1**	**0**	**+1**	**+α**
The concentration (N) of alkaline NaOH pretreatment	X_1	0.58	0.75	1	1.25	1.42
The concentration (N) of acid HCl pretreatment	X_2	0.08	0.25	0.5	0.75	0.92
Extraction temperature (°C)	X_3	33.2	40	50	60	66.8

The gelatin extraction process based on [10] method with adaptations. The raw materials (200 g) were soaked in a solution of NaOH (1: 5 w/v) with various concentrations (factor X_1, N) for 1 hour. Then, the samples were washed with water (1: 5 w/v) three times, filtered and then squeezed. Subsequently, the samples were immersed in HCl (1: 5 w / v) with various concentrations (factor X_2, N) for 1 hours, then the samples were washed with water (1: 5 w/v) three times, filtered and then squeezed. After that, the samples were put into glass beakers, added with distilled water (1: 4 w/v) and covered with aluminum foil. Subsequently, the samples were extracted using a water bath with various extraction temperatures (factor X_3, °C) for 3 hours. Next, the filtrate was obtained by filtering using cheesecloth and dried in a glass container at a temperature of 60°C for 72 hours.

Table 2. Central composite rotatable design with 3 factors (X) and 5 levels of independent variables, along with dependent variables (Y).

Run	X_1[a] (N)	X_2[b] (N)	X_3[c] (°C)	Y_1 = Yield (%)		Y_2 =Emulsion activity index (m²/g)		Y_3 =Gel strength (g)
				Exp.	**Pred.**	**Exp.**	**Pred.**	**Exp.**
1	0	0	0	6	5,81	32,08	38,95	236,7
2	-α	0	0	8,87	7,51	19,36	22,48	189,3
3	0	-α	0	5,45	5,13	40,48	34,6	53,83
4	0	0	0	5,44	5,81	19,84	28,52	127,7
5	0	0	-α	6,14	8,32	17,65	18,39	218,2

6	-1	1	-1	5,09	5,73	19,58	21,91	138,8
7	0	0	0	6,13	5,81	27,19	28,52	174
8	0	0	0	5,8	5,81	22,39	28,52	297,7
9	α	0	0	4,3	4,11	23,88	14,5	269,7
10	-1	-1	1	9,06	7,91	31,51	32,89	119
11	1	1	-1	3,5	3,71	31,45	34,49	48,33
12	0	0	-α	3,83	3,3	31,71	24,71	198
13	0	α	0	6,17	6,49	39,34	30,81	133,3
14	-1	-1	-1	3,58	4,92	25,5	25,6	10,5
15	-1	1	1	9,5	8,72	36,28	30,81	9,5
16	1	1	1	8,25	6,7	15,35	19,68	3,833
17	1	-1	-1	3,76	2,9	17,34	27,23	66,5
18	0	0	0	4,92	5,81	34,42	28,52	140
19	0	0	0	4,83	5,81	34,12	28,52	109,7
20	1	-1	1	5,62	5,88	8,715	10,81	2,833

[a]The concentration of pretreatment alkaline NaOH (N)
[b]The concentration of acid HCl pretreatment (N)
[c]The extraction temperature
[d] Experiment
[e] Prediction

3. Results and Discussion

3.1. Response Surface Model Building of Gelatin Extraction

The response surface models for every response investigated showed in Table 3. Based on the results, in the optimization of the gelatin extraction, the response variables optimized were yield, emulsion activity index, and gel strength.

Table 3. Response surface models of tilapia gelatin extraction.

Response	Model	P- Value		Lack of fit		R^2
Yield	Linear	<0.0001	Significant	0.0544	Non significant	0.7375
Emulsion activity index	Quadratic	0.1723	Non significant	0.2655	Non significant	0.6269
Gel strength	Mean	-	-	0.2814	Non significant	-

3.2. Yield

The bone and skin tilapia gelatin yield ranged from 3.49% to 9.5%. Generally, the average of fish gelatin yield is 6-19% [11]. Response surface model of tilapia gelatin yield was linear (Table 3). The RSM equation of the gelatin extraction optimization towards the yield response is: Yield = +5,81-1,01A+0,41B+1,49C, where A= the concentration of alkaline pretreatment, B= the concentration of acid pretreatment, and C= the extraction temperature. The yield response will increase proportionally with the decrease of alkaline pretreatment concentration, and the increase of acid pretreatment also extraction temperature which are indicated by negative and positive value, respectively. This presumably because acid produced a pH that was suitable for the extraction of gelatin. Acid and temperature increase also caused some cross-linkages easily to break with little damage to the polypeptide chain [12]. Therefore, a higher yield could be generated.

Figure 1 and 2 show surface shape on three-dimensional graph of interaction among components (X_1, X_2, X_3) interplay yield response values. Different colors on the graph indicate different yield values. Blue indicates the lowest yield response value and red indicates the highest yield response. The lines which composed of dots on the graph represent the combination of three components with different values which produced similar yield response. Figure 1 and 2 showed that yield increase at combination of lower alkaline concentration, and higher acid concentration and extraction temperature. Kittiphattanabawon et al., 2016 [13] was using only 0.1-3% HCl in the pretreatment process with 40-80^{o}C extraction temperature stated that gelatin extracted at higher temperature for longer time (>12 hours) had higher yield but poorer gel strength. However, Niu et al., 2013 [14] indicated that adjusting acids concentrations to appropriate levels resulting in optimal protein yield and gelatin physicochemical properties.

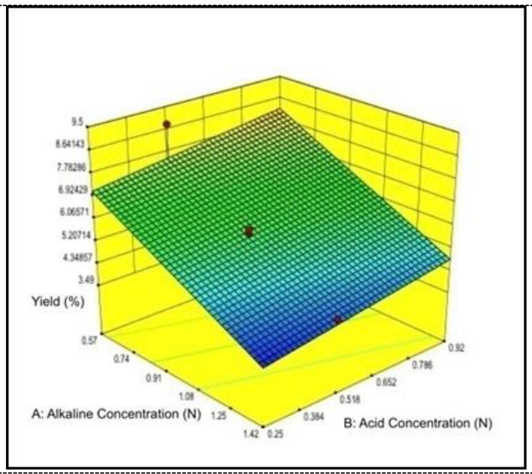

Figure 1. Surface profile plot of yield response to alkaline and acid concentrations.

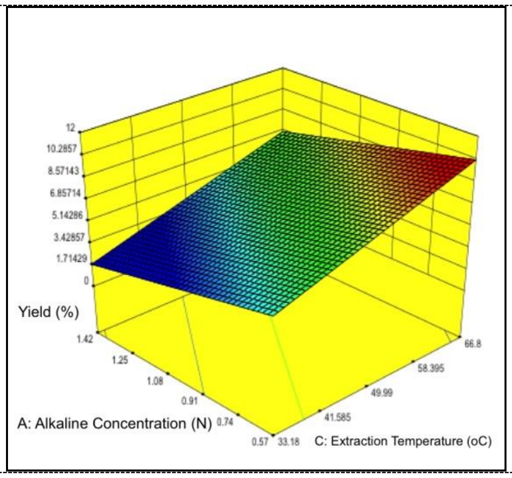

Figure 2. Surface profile plot of yield response to alkaline concentration and extraction temperature.

3.3. Emulsion Activity Index (EAI)

Tilapia gelatin EAI ranged from 8,7-40.47 m2/g. Gelatin as an emulsifier is usually applied on the products such as cream soup, sauces, flavorings, meat pastes, and whipped cream. The tilapia EAI response surface model suggested was quadratic (Table 3). The RSM equation of the gelatin extraction optimization towards the EAI response is: $EAI= 28,52-2,37A+1,29B- 1,88C+2,74AB-5,93AC+0,4BC-3,55A^2+ 2,92B^2- 2,46C^2$. Figure 3 shows that the EAI increase as the alkaline concentration decrease and the acid concentration increase. On the other hand, the EAI also increase as the extraction temperature decrease when the alkaline concentration decrease or the acid concentration increase (Figure 4 and 5).

3.4. Gel Strength

The gel strength of commercial gelatin range between 50-300 g [9]. The skin and bone tilapia gelatin have gel strength between 2.83-297.67 g, which suitable for product such as wafers, dairy products, desserts, and marshmallow. Depend on fish habitats, gelatin of other fish such as cold water salmon has a gel strength of 195 g [15], while warmer water cat fish at 252 g [16]. Furthermore, da Silva et al., 2017 [17] was using NaOH, acetic acid and kumakuma (Brachyplatystoma filamentosum) fish skin from the Amazonian industries produced gelatin with high yield and gel strength, 19.7% and 244.3g. Response surface model of tilapia gelatin gel strength was mean (Table 3 and Figure 6).

IOP Conf. Series: Materials Science and Engineering **334** (2018) 012061 doi:10.1088/1757-899X/334/1/012061

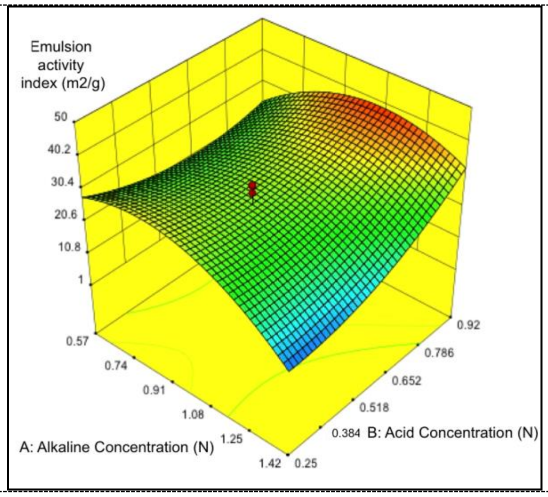

Figure 3. Surface profile plot of EAI response to alkaline and acid concentrations.

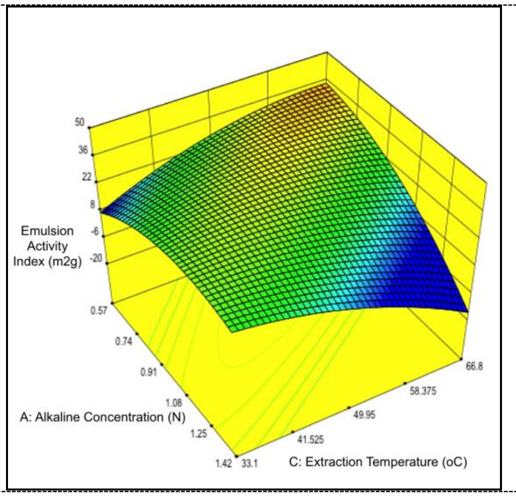

Figure 4. Surface profile plot of EAI response to alkaline concentration and extraction temperature.

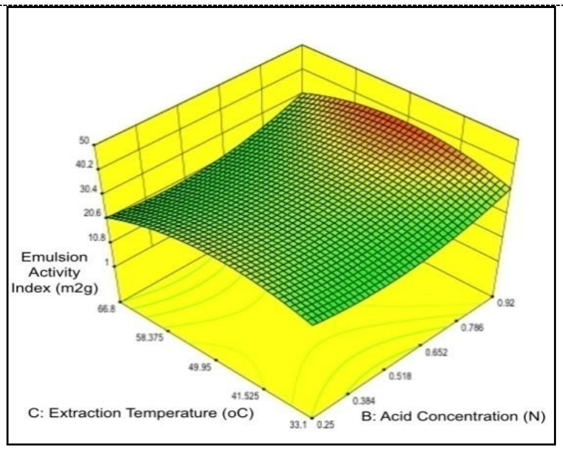

Figure 5. Surface profile plot of EAI response to acid concentration and extraction temperature.

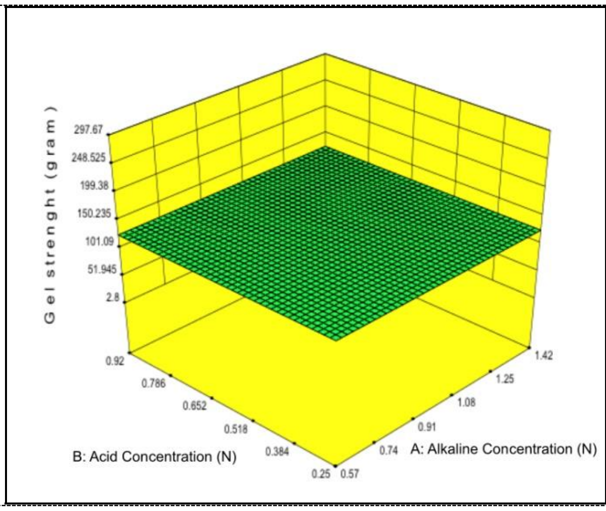

Figure 6. Surface profile plot of gel strength response to alkaline and acid concentration (N).

3.4. Gelatin Extraction Optimization

The purpose of optimization was to minimize the effort required and to maximize the desired results. Through this approach, optimization of multi responses could be obtained using desirability functions. Table 4 shows the components which were optimized, targeted, limits, as well as the level of importance at the stage of formulating the optimization. The concentration of alkaline and acid, and extraction temperature will affect the quality of the resulting gelatin. Since yield, emulsion activity index (EAI), and gel strength responses have the highest level of importance (5=+++++), therefore only these responses that would be optimized.

Table 4. Optimization parameters in the response optimizer.

Component/ Response	Target	Lower Limit	Upper Limit	Importance[a]
Alkaline Concentration	In range	0.75	1.25	3(+++)
Acid Concentration	In range	0.25	0.75	3(+++)
Extraction Temperature	In range	40	60	3(+++)
Yield	Maximum	3.50	3.50	5(+++++)
Emulsion Activity Index (EAI)	In Target	8.72	40.48	5(+++++)
Gel Strength	In Target	2.83	297.70	5(+++++)

[a]Importance level range from 1 to 5 with 1 as the lowest importance and 5 as the highest importance

There were 33 optimization solution formulas obtained from the optimization process which had the desirability values all over 0.91, except one with the lowest value of 0,89. Gelatin extraction process conditions recommended was using alkaline (NaOH) pretreatment concentration of 0.77 N, acid (HCl) pretreatment concentration of 0.59 N, and gelatin extraction temperature of 66.79°C. The optimal solution formula had optimization targets of 94.38% and was predicted to produce gelatin with yield of 9.4%, emulsion activity index of 26.97 m^2/g, and gel strength of 120.96 g.

4. Conclusion

The decrease in alkaline pretreatment concentration combine with the increase of extraction temperature cause an increase in yield of the extracted tilapia gelatin. On the other hand, the decrease of alkaline concentration and extraction temperature resulted in high emulsion activity index (EAI). There is no significant effect of alkaline and acid pretreatment concentration, and gelatin extraction temperature on gel strength of the extracted tilapia gelatin. Gelatin yield were 3.49-9.5%, while EAI and gel strength were 8.71-40.47 m2/g and 2.83-297.67 g, respectively. Optimization using expert design program 9.0.5 via response surface methodology approach generates optimal formula for gelatin extraction with alkaline concentration (NaOH) of 0.77 N, acid (HCl) of 0.59 N and temperature of 66.80°C with desirability value of 94.38% and was predicted to produce gelatin with 9.40% of yield, gel strength of 127.37 g, and emulsion activity index of 26.97 m2/g.

Acknowledgment
We thank Syiah Kuala University, Banda Aceh and Kemenristekdikti Indonesia “Fundamental Research Project”, for financial support provided.

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