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Optimization of Gelatin Extraction from the Skin of Freshwater Carps by Response Surface Methodology

George Ninan, Jose Joseph, A.A. Zynudheen, P.T. Mathew and V. Geethalakshmi
Central Institute of Fisheries Technology,
Kochi - 682 029, India

Response Surface Methodology (RSM) was used to determine the optimum operating conditions for extracting the gelatin from the skin of three species of freshwater carps viz., Rohu(*Labeo rohita*), Common Carp (*Cyprinus carpio*) and Grass Carp (*Ctenopharyngodon idella*). From the screening experiments, four independent variables viz., alkali pretreatment concentration, acid pretreatment concentration, pretreatment time and extraction temperature were identified as the critical variables that had significant effect on the extraction of gelatin from the skin of these species. The responses studied were gel strength and yield. The optimum gelatin extraction conditions from the skin of three species based on the responses were 51.35 minutes (pre-treatment time) and 49.33^{0}C (extraction temperature) for Rohu skin; 60 minutes (pre-treatment time) and 57.97^{0}C (extraction temperature) for Common Carp skin; 60 minutes (pre-treatment time) and 40^{0}C (extraction temperature) for Grass Crap skin. The gelatin from the skin of the freshwater carps showed high yield, medium gel strength and high viscosity.

Key words: Response Surface Methodology, optimization, Rohu, Common Carp, Grass Carp, skin, gelatin, extraction, gel strength, yield

Gelatin has wide applications in the food and pharmaceutical industries. Most of the commercial gelatins are derived from mammalian sources, mainly pigskin and cowhide. Gelatin from marine sources is a possible alternative to bovine gelatin (Kim & Mendis, 2006; Rustad, 2003; Wasswa *et al.*, 2007). Fish skin contains large amounts of collagen and can be considered as a potential source of gelatin. One major advantage of gelatin from aquatic sources is that it is not associated with the risk of Bovine Spongiform Encephalopathy and is acceptable to most religious groups. Further, the utilisation of fish skin for the extraction of gelatin can significantly address the problem of waste disposal in the fish processing industry. Although fish gelatin will be unable to completely replace mammalian gelatin, in future it might become a niche product offering unique and competitive properties to other biopolymers, as well as meeting the demand of global halal/kosher market (Karim and Bhat, 2009). Extraction of fish gelatin has been reported from many species viz cod (Gudmundsson and Hafsteinsson 1997), hake (Montero *et al.*, 1999), megrim (Gomez-Guillen & Montero 2001), tilapia (Jamilah and Harvinder 2002), yellowfin tuna (Lefebvre *et al.*, 2002; Cho *et al.*, 2005), Alaska pollock (Zhou and Regenstein 2004, 2005) catfish (Yang *et al*, 2007) Nile Perch (Muyonga *et al.*, 2004) and Big eye snapper (Binsi *et al.*, 2009). Recent studies indicate that gelatins from the skin of warm water species have functional properties comparable to that of mammalian gelatins (Cho *et al.*, 2005).

Response Surface Methodology (RSM) is a mathematical modeling technique that relates product treatment to outcomes and establishes regression equations that describe inter-relations between input parameters and product properties (Rao, *et al.*, 2000; Ozdemir & Devres, 2000). The basic principle of RSM is to determinate model equations that describe interrelations between the independent variables and the dependent variables (Edwards & Jutan, 1997). Single step optimization approach was

reported for the optimization of fish skin gelatin extraction from several fish species (Gudmundsson & Hafsteinsson 1997; Cho *et al* 2004, 2005, 2006; Zhou and Regenstein 2004.).

The present study is aimed at optimizing the extraction of gelatin from the skin of three commercially important freshwater carp species and assessing its physico-chemical properties.

Materials and Methods

The raw materials for the study were the skins of three cultured freshwater fishes viz., Rohu (*Labeo rohita – Hamilton Buchanan)* Common Carp (*Cyprinus carpio*) and Grass Carp (*Ctenopharyngodon idella*). Fish samples were procured from a local fish farm and brought to the laboratory in iced condition. The average length and weight of the samples were: Rohu – 55 cm & 2500 g, Common carp - 30 cm & 1500 g and Grass carp - 60 cm & weight 2600 g.

The samples were filleted and the skins were manually removed. They were then cleaned by removing the scales, washed and stored at -18^{0}C with a maximum storage of less than two months before use.

The method of Grossman and Bergman (1992) was used for the extraction of gelatin from fish skin. Frozen skins were thawed at 4^{0}C overnight, chopped into pieces of 2 to 3 cm, and washed with tap water in the ratio 1: 6 w/v for 10 min for 3 times. The cleaned skins were drained using cheesecloth for 5 minutes and the cheesecloth containing the skins were squeezed manually to remove water.

Cleaned skin was treated with sodium hydroxide in the ratio 1:6 w/v for variable times (Table 1). The samples were rinsed with tap water, drained then treated with sulphuric acid in 1:6 w/v for variable times (Table 1), rinsed with tap water and drained using cheesecloth. The acid and alkali treatment was repeated two times. The pretreated samples were transferred into conical flasks and placed in water bath with varying volumes of deionized water for variable times and temperatures (Table 1). The clear extract obtained was filtered in a Buchner funnel using Whatman filter paper No.4 and concentrated in a Rotary Evaporator (IKA RV06 –Germany), freeze dried (Martin Christ, Gamma 1-16 LSC- Germany) and stored.

Table 1. Pretreatment conditions for the extraction of gelatin from carp skin

Process variables	Range		
NaOH concentration	0.1M	0.15M	0.2M
H_2SO_4 concentration	0.1M	0.15M	0.2 M
Pretreatment time	40Min	50 Min	60Min
Skin/water ratio	1:4	1:5	1:6
Extraction time	6H	8H	10H
Extraction temperature	40^{0}C	50^{0}C	60^{0}C

Process Optimization

Screening experiments were carried out to determine the critical variables for the extraction of gelatin with a Fractional Factorial Design, where an appropriately chosen small fraction of the full factorial design permits the study of a large number of variables in an economical number of trials. The Fractional Factorial Design used in the present study is a resolution three design ($2^{6-3/iii}$) in which the main effects are confounded with two factor interactions (Tables 2 & 3).

After the important variables were determined by screening, Response Surface Methodology was used for optimizing gelatin extraction. Four factors were identified as critical variables. (Table 4). Based on the results, a Central Composite Rotatable Design was formulated (Table 5) and two responses viz., gel strength and yield were evaluated (Design-Expert 6.0.11, Stat-Ease, Inc., Minneapolis MN, USA).

The yield was calculated as described by Muyonga *et al.*, (2004).

Table 2. Independent variables and their levels in the 6 factor, 2 level fractional factorial ($2^{6-3/iii}$) screening design*

Independent variables	Symbol	Levels		
		-1	0	+1
NaOH concentration	X1	0.1M	0.15M	0.2M
H_2SO_4 concentration	X2	0.1M	0.15M	0.2 M
Pretreatment time	X3	40Min	50 Min	60Min
Skin/water ratio	X4	1:4	1:5	1:6
Extraction time	X5	6H	8H	10H
Extraction temperature	X6	40°C	50°C	60°C

*No. of variables: 6 , Levels: 2, Observations : 8, Resolution : 3,Wt. of sample : 30g for each run , Pretreatment ratio 1: 6

$$\text{Yield, \%} = \frac{C \times V \times 100}{M}$$

Where C = concentration of light liquor, g/ml, V = liquor volume, M = weight of skin sample (g) used for extraction. The gel strength was determined by the British Standard 757: 1975 method (BSI, 1975) using a texture analyzer (Lloyd Instruments, Model *LRX* Plus, U.K.). Moisture, protein, fat and ash contents of the extracted gelatins were determined by AOAC (1995) methods. For protein determination, nitrogen conversion factor of 5.4 was used (Eastoe & Eastoe, 1952). The pH of gelatin solution was measured by the method of BSI 757 (1975). Colour analysis of the sample was performed with Hunter lab Miniscan ® XE plus Spectrocolorimeter (Hunter Associates Laboratory, Inc. Reston, Virginia, USA). Measurements were recorded using the L* a* b* colour scale (CIE, 1986). Viscosity (cP) of 10 ml of the Gelatin solution of 6.67% (w/v) was determined using Brookfield Digital Viscometer (Model DV E Brookfield Engineering, USA) equipped with a No.1 spindle at 30 ± 0.5°C (Cho *et al.*, 2006).

All data were analysed for the Analysis of Variance (ANOVA) and Duncan's Multiple Test were carried out to determine the significance of difference between the means. Statistical package used in the study was SAS, Version 6 (1989). All data represented are the means of triplicates.

Results and Discussion

The six important factors (independent variables) that affect the extraction of gelatin from fish skin and their ranges between model levels described as –1 and +1 were selected for the screening experiments. The importance of these factors was evaluated based on the responses on two dependent variables selected, viz., gel strength (Bloom) and yield (%). They are rated as the most

Table 3. Fractional factorial screening design in coded units

Standard Order	X1	X2	X3	X4	X5	X6
1	-1	-1	-1	+1	+1	+1
2	+1	-1	-1	-1	-1	+1
3	-1	+1	-1	-1	+1	-1
4	+1	+1	-1	+1	-1	-1
5	-1	-1	+1	+1	-1	-1
6	+1	-1	+1	-1	+1	-1
7	-1	+1	+1	-1	-1	+1
8	+1	+1	+1	+1	+1	+1

Table 4. Critical variables and their levels in the 4 factor, 5 level Central Composite Rotatable Design for optimization of the extraction conditions of gelatin from carp skin

VARIABLES	SYMBOL		CODE LEVEL				
	coded	uncoded	-2	-1	0	1	2
NaOH concentration (mol/L)	X1	X1	0.05	0.1	0.15	0.2	0.25
H_2SO_4 concentration (mol/L)	X2	X2	0.05	0.1	0.15	0.2	0.25
Pretreatment time (minutes)	X3	X3	30	40	50	60	70
Extraction temperature (^{0}C)	X4	X4	30	40	50	60	70

commercially important physical properties of the extracted gelatin. A total of eight groups of extraction experiments were conducted using different combinations of these six factors and the responses are shown in Table 6.

The range of Responses for independent factors derived from the Screening Experiment for carp Skin Gelatin is given in Table 7. From this data, four factors were identified as critical variables that had a significant effect on the extraction of gelatin. They were alkali pretreatment concentration (mol/L), acid pretreatment concentration (mol/L), pretreatment time (min) and extraction temperature (^{0}C) and designated as coded units X_1, X_2, X_3 & X_4 respectively. Other factors were set on the basis of the preliminary experiment with an extraction time of eight hours and the skin/water ratio of 1:5 for all the experiments conducted thereafter.

The screening experiments provide the information about the steps that are crucial for the efficient extraction. The degree of conversion of collagen into gelatin (yield) and gel strength are related to the severity of the pretreatments viz., alkali and acid pretreatment, pretreatment time and the extraction temperature (Montero and Gomez-Guillen 2000; Yang *et al*, 2007).

Experimental results of the 4 factor, 5 level Central Composite Design are shown in Tables 8 to 10. The Quadriatic Response Surface Analysis was based on Multiple Linear Regression taking into account of all main, quadriatic and interaction effects. The predicted values calculated are listed together with the experimental data. The

Table 5. Central Composite Design for Optimizing the Extraction Conditions of Carp Skin Gelatin

STANDARD ORDER	X1	X2	X3	X4
01	-1	-1	-1	-1
02	1	-1	-1	-1
03	-1	1	-1	-1
04	1	1	-1	-1
05	-1	-1	1	-1
06	1	-1	1	-1
07	-1	1	1	-1
08	1	1	1	-1
09	-1	-1	-1	1
10	1	-1	-1	1
11	-1	1	-1	1
12	1	1	-1	1
13	-1	-1	1	1
14	1	-1	1	1
15	-1	1	1	1
16	1	1	1	1
17	-2	0	0	0
18	2	0	0	0
19	0	-2	0	0
20	0	2	0	0
21	0	0	-2	0
22	0	0	2	0
23	0	0	0	-2
24	0	0	0	2
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0
31	0	0	0	0

Table 6. Experimental Results for Carp Skin Gelatin Extraction using Fractional Factorial Screening Design (2^{6-3} /iii) in coded units*

	Independent variables						Dependent variables					
							Rohu		Common carp		Grass carp	
No.	X1	X2	X3	X4	X5	X6	Gel strength (B)	Yield (%)	Gel strength (B)	Yield (%)	Gel strength (B)	Yield (%)
1	-1	-1	-1	+1	+1	+1	123.29 (1.83)	13.60 (0.39)	100.43 (2.54)	11.32 (3.09)	80.74 (5.02)	7.51 (0.30)
2	+1	-1	-1	-1	-1	+1	219.04 (2.79)	14.61 (0.38)	203.51 (2.39)	13.03 (0.99)	239.87 (1.68)	11.03 (0.25)
3	-1	+1	-1	-1	+1	-1	207.05 (3.56)	14.18 (1.68)	207.26 (4.67)	9.70 (2.59)	110.10 (1.32)	8.43 (0.47)
4	+1	+1	-1	+1	-1	-1	202.70 (2.39)	14.05 (0.84)	200.97 (2.72)	14.71 (0.73)	211.63 (2.62)	10.27 (1.96)
5	-1	-1	+1	+1	-1	-1	200.39 (2.72)	10.75 (1.19)	202.51 (4.32)	12.49 (0.47)	234.83 (3.96)	10.75 (0.96)
6	+1	-1	+1	-1	+1	-1	189.71 (2.17)	7.32 (1.06)	189.05 (2.62)	12.36 (0.49)	256.46 (3.03)	12.42 (0.72)
7	-1	+1	+1	-1	-1	+1	172.13 (1.53)	13.74 (0.60)	160.68 (1.88)	12.75 (0.66)	163.37 (9.14)	10.39 (0.38)
8	+1	+1	+1	+1	+1	+1	119.83 (1.15)	15.34 (1.37)	125.83 (4.06)	12.95 (0.45)	113.45 (1.56)	9.10 (0.08)

*Values in brackets are standard deviations of triplicate samples. Independent variables and their ranges X_1: Alkaline concentration, 0.1 to 0.2 mol/L; X_2: Acid concentration, 0.1 to 0.2 mol/L :X_3 pretreatment time, 40 to 60 min; X_4: Skin/water ratio, 1/4 to 1/6 w/w. X_5: Extraction time, 6H to 10H X_6: Extraction temperature, 40 to 60 °C

Analysis of Variance for the Response Surface model is given in Table 11. Since the experimental design had seven replicate runs at the centre point, the residual sum of squares was partitioned between pure error and lack of fit components. The *p* values for the lack-of-fit test were large which indicated that the quadriatic models were adequate. The *p* values for the significance of regression were very small indicating that at least some of the parameters in the models were not zero. For all the responses, both linear and quadriatic terms contributed significantly to the models. Interaction did not contribute significantly for both responses. The values of R^2 suggest that the models can explain a high percentage of the variability in the observed data. Thus the analysis of variance shows the predicted models are statistically valid.

For determining the overall optimum conditions in a multi response situation requires the use of desirability functions and in this study the optimization method developed by Derringer and Suich (1980) and described by Myers and Montgomery(2002) was used. Here a one sided desirability function was used with the responses to be maximized. The programme used five possibilities for a goal to construct the desirability indices viz., maximum, minimum, in target, in range and is equal. Table 12 lists the optimization parameters for the independent factors and responses. Among the independent factors the goal for alkali and acid concentration to be used in the process is set as minimum and for the other two factors the goal is set in range. For the responses the goal is set as maximum. The limits for each goal were set by the software based on the response surface model constructed in the previous section. The parameter called weights; gives added emphasis to upper and lower limits or emphasizes a target value. Here the weights

Table 7. Range of Responses for independent factors derived from the Screening Experiment for Carp Skin Gelatin Extraction

	Level	X_1	X_2	X_3	X_4	X_5	X_6
				Rohu			
Gel	-1	803.59	787.6	761.09	702.54	732.42	808.87
Strength	+1	639.56	655.55	682.06	740.61	710.73	634.28
	Range	164.03*	132.05*	79.03*	38.07	21.69	174.59*
	-1	49.85	46.27	56.43	53.15	52.26	46.3
Yield	+1	53.73	57.31	47.15	50.43	51.32	57.28
	Range	3.88	11.04*	9.28*	2.72	0.94	10.98*
				Common Carp			
Gel	-1	667.21	756.83	796.13	708.51	695.51	796.05
Strength	+1	719.37	629.75	590.45	678.07	691.07	590.53
	Range	52.16	127.08*	205.68*	30.44	4.44	205.52*
	-1	46.26	47.84	48.76	49.26	49.2	52.99
Yield	+1	53.05	51.47	50.55	50.05	50.10	46.32
	Range	5.9*	3.63*	1.79	0.79	0.9	6.67*
				Grass Carp			
Gel	-1	584.38	794.24	816.70	604.68	744.14	786.36
Strength	+1	775.08	565.21	542.75	754.78	615.31	573.10
	Range	190.70*	229.03*	273.95*	150.10	128.83	213.26*
	-1	37.09	42.28	37.25	41.72	41.88	42.45
Yield	+1	42.82	37.64	42.67	45.40	38.04	37.47
	Range	5.73*	4.64	5.42*	3.68	3.84	4.98*

* Indicates significant ($P < 0.05$) differences among the 2 levels.

are given as one, with which the desirability will vary from zero to one in a linear fashion. Importance is a relative scale for weighing each of the resulting desirability in the overall desirability of the final product. Hence the importance is set as three for all the factors. A desirability value near to one is good.

The resultant solutions obtained using the response optimizer is given Table 13. The optimization solutions for all the three products give a composite desirability value above 0.8 based on the set parameters. The responses predicted by the solutions are within the range of the experimental values obtained in the response surface model. Higher values for responses could be obtained by altering the goal of the independent factors particularly alkali and acid concentrations used in the process.

The response surface plots based on the above optimization is illustrated in Figs 1 to 6. Since alkali and acid concentrations had the most significant effects on the responses, the response surface plots were set with other two factors viz., pretreatment time and extraction temperature at the median values of the lower and upper limits i.e., 50 min and 50^0C to determine the interaction of alkali and acid concentration. In the case of extraction of rohu gelatin, increase in the concentration of NaOH and H_2SO_4 results in the increase in gel strength and the effect was more pronounced in the case of the change in NaOH concentration (Fig.1). The influence of these factors on yield shows the reverse trend (Fig. 2). For common carp gelatin, the same trend can be observed (Figs 3 & 4). It can be seen that increase in gel strength and yield was significantly influenced by the concentration of NaOH only

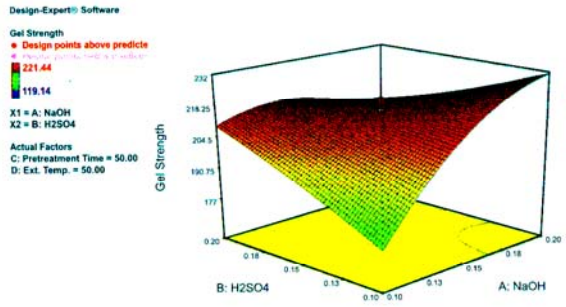

Fig. 1. Three-dimensional response surface plot - Rohu skin gelatin based on gel strength

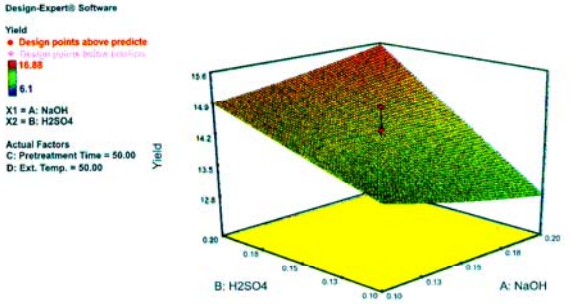

Fig. 2. Three-dimensional response surface plot - Rohu skin gelatin based on yield

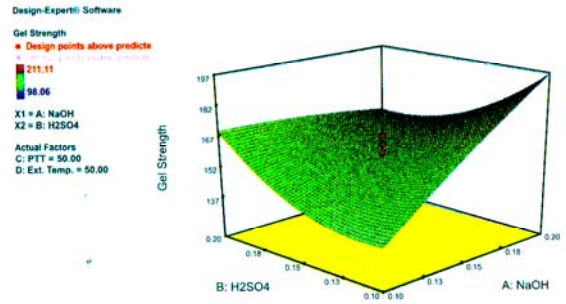

Fig. 3. Three-dimensional response surface plot - Common carp skin gelatin based on gel strength

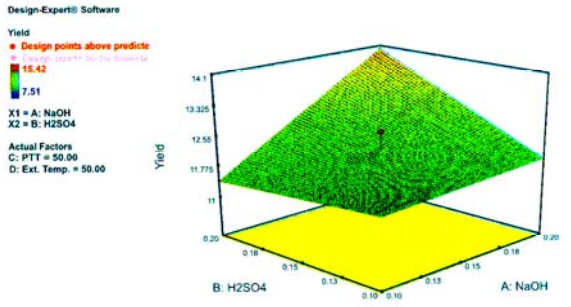

Fig. 4. Three-dimensional response surface plot - Common carp skin gelatin based on yield

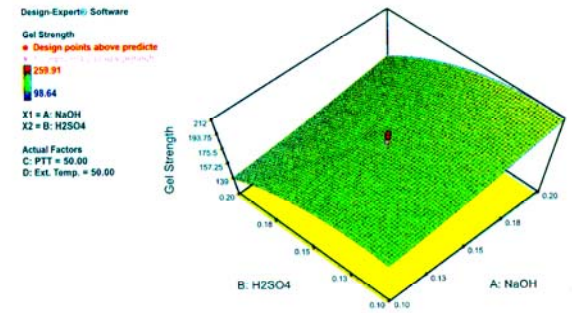

Fig. 5. Three-dimensional response surface plot - Grass carp skin gelatin based on gel strength

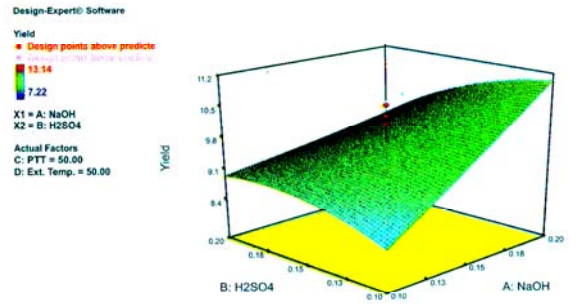

Fig. 6. Three-dimensional response surface plot - Grass carp skin gelatin based on yield

under the given set of optimization parameters (Figs. 5 & 6) for the extraction of grass carp gelatin. The results suggest that alkali concentration is the most important factor affecting the gel strength and yield in all the three extraction processes under a given set of optimization parameters. Zhou and Regenstein (2004) observed that higher acid concentration resulted in lower gel strength during the extraction of Pollock skin gelatin whereas too low or too high acid concentration resulted in poor yield. It was observed that the acid pretreatment for the extraction of gelatin from grass carp, increase in the concentration of HCl caused increase in yield and gel strength up to a point after which the increase in acid concentration resulted in the decrease of the above factors (Kasankala *et al.*, 2007). Higher acid concentration produces gelatin with

Table 8. Central Composite Design for Rohu skin gelatin extraction with experimental data and predicted values

STANDARD ORDER	Gel Strength (B)		Yield (%)		STANDARD ORDER	Gel Strength (B)		Yield (%)	
	Expt.	Pred.	Expt.	Pred.		Expt.	Pred.	Expt.	Pred.
01	123.12	129.39	13.37	14.91	16	119.19	127.64	16.88	17.17
02	215.98	212.93	14.83	15.92	17	125.2	117.18	13.37	12.82
03	208.71	212.83	13.56	12.86	18	221.44	225.14	14.18	13.59
04	214.94	218.14	13.11	12.74	19	202.41	200.62	12.89	12.86
05	201.07	206.43	11.27	12.06	20	220.99	218.14	14.31	15.16
06	192.02	197.34	7.79	7.28	21	202.7	194.28	9.39	9.43
07	170.83	163.80	13.47	12.55	22	189.38	199.95	6.1	5.37
08	121.15	127.65	14.25	13.95	23	173.82	184.37	13.32	14.10
09	121.55	117.18	14.05	14.91	24	119.14	126.71	14.88	14.20
10	219.69	212.93	14.83	15.92	25	205.62	215.60	14.03	14.09
11	209.47	212.62	16.08	15.49	26	221.44	215.60	14.83	14.09
12	200.18	205.93	14.73	15.36	27	220.99	215.60	13.11	14.09
13	197.39	194.28	11.59	12.06	28	219.69	215.60	14.83	14.09
14	187.72	182.39	8.06	9.53	29	200.18	215.60	14.73	14.09
15	171.74	178.76	14.42	14.68	30	215.98	215.60	14.18	14.09
					31	214.94	215.60	14.31	14.09

Table 9. Central Composite Design for Common carp skin gelatin extraction with experimental data and predicted values

STANDARD ORDER	Gel Strength (B)		Yield (%)		STANDARD ORDER	Gel Strength (B)		Yield (%)	
	Expt.	Pred.	Expt.	Pred.		Expt.	Pred.	Expt.	Pred.
01	103.12	105.34	9.09	9.21	16	128.31	130.73	12.67	13.06
02	205.18	208.41	14.01	15.13	17	98.06	105.33	10.01	11.31
03	211.11	208.49	7.51	7.59	18	204.59	208.49	12.04	13.03
04	203.84	205.88	14.75	14.71	19	200.07	198.68	9.03	9.69
05	199.1	197.61	12.86	12.49	20	200.64	205.87	13.96	14.71
06	192.02	184.05	11.98	12.08	21	207.37	207.42	11.96	12.49
07	159.33	167.08	12.01	12.48	22	187.05	195.54	12.91	12.95
08	121.15	118.72	13.46	13.06	23	159.88	151.38	13.29	13.38
09	100.12	105.33	14.85	13.41	24	128.03	136.52	12.71	12.76
10	200.77	198.61	13.05	13.03	25	156.72	160.21	12.11	12.08
11	199.59	198.68	12.55	11.79	26	150.90	160.21	11.89	12.08
12	198.44	196.07	15.42	14.71	27	167.34	160.21	12.67	12.08
13	201.07	207.41	12.66	12.49	28	153.01	160.21	12.01	12.08
14	188.09	196.06	12.18	12.08	29	162.67	160.21	11.52	12.08
15	162.82	167.08	12.95	12.48	30	158.88	160.21	11.04	12.08
					31	160.11	160.21	11.91	12.08

shorter fragments negatively affecting the gel strength. Extraction temperature is the most important factor that affect the responses in the optimization of extraction of gelatin from the skin of yellow fin tuna (Cho *et al* 2005).

Verification experiments were conducted under optimal conditions to compare

predicted values and actual values of responses (Table 14). Both actual values and predicted values were almost same and hence the values estimated by response surface model were adopted for optimization of gelatin extraction.

The proximate compositions of gelatins are given in Table 15. Generally, the skin gelatins of the three species showed high values for proteins and low values for moisture and fat. Grass carp gelatin contained significantly higher content of protein ($p < 0.05$) than the other two gelatins. Jongjajareonarak *et al.*, (2006) reported a protein content of 87.9% & 88.6% for freeze dried gelatin from the skin of big eye snapper and brown eye snapper respectively. Freeze-dried gelatin from the skin of adult Nile perch contained 88% protein when extracted at 50°C (Muyonga *et al*, 2004).

Moisture content of the samples was below 10% which was less than the limit prescribed for edible gelatin i.e., 15% (GME, 2008). Freeze drying of the gelatin samples was the reason for very low moisture content. The moisture content of gelatin may be as high as 16%, however, normally it is around 10 -13%. At 13% moisture, the glass transition temperature of gelatin is about 64°C which allows particle size reduction to be a simple operation (McCormick, 1987). In addition, at 13% moisture content and 25°C gelatin is close to equilibrium with ambient air moisture contents of ca. 46% RH. At 6 to 8% moisture, gelatin is very hygroscopic and it becomes difficult to determine the physical attributes with accuracy (Cole 2000).

The ash content in the three samples were in the range of 1.10 - 1.18%, much less than the recommended maximum limit of 2.6% (Jones 1977) and the limit set for edible gelatin (2%) (GME, 2008). The differences in mineral contents between the skins of the species might be associated with the varying ash contents obtained.

The pH of gelatins varied between 4.05 and 4.42 (Table 15). Grass carp gelatin showed significantly higher pH ($p < 0.05$) than the other two gelatins. The pH was

Table 10. Central Composite Design for Grass carp skin gelatin extraction with experimental data and predicted values

STANDARD ORDER	Gel Strength(B)		Yield(%)		STANDARD ORDER	Gel Strength (B)		Yield(%)	
	Expt.	Pred.	Expt.	Pred.		Expt.	Pred.	Expt.	Pred.
01	88.12	82.41	7.51	6.88	16	103.1	109.12	9.19	9.14
02	241.39	240.96	11.29	11.03	17	78.71	82.40	7.81	7.51
03	111.22	103.77	8.22	8.43	18	201.16	203.83	10.79	11.03
04	240.44	233.80	11.71	10.27	19	101.45	103.77	8.11	8.43
05	234.11	234.83	11.79	10.75	20	134.18	134.62	8.04	9.14
06	255.28	257.59	13.14	12.78	21	239.1	234.83	9.89	9.62
07	173.37	167.33	10.77	10.58	22	244.2	245.67	11.7	12.04
08	115.15	109.12	9.09	9.14	23	155.44	157.10	10.01	11.42
09	80.39	82.47	7.22	7.51	24	192.09	198.22	9.03	10.20
10	229.07	223.87	11.01	11.03	25	191.09	188.06	9.28	9.44
11	98.64	103.77	8.97	8.43	26	180.22	188.06	10.54	9.44
12	209.26	207.94	11.06	10.27	27	195.31	188.06	9.01	9.44
13	231.28	234.83	10.58	10.75	28	191.11	188.06	9.27	9.44
14	259.91	257.59	12.42	12.78	29	178.02	188.06	10.11	9.44
15	161.3	167.33	10.4	10.58	30	185.50	188.06	10.29	9.44
					31	186.21	188.06	9.89	9.44

Table 11. Carp skin gelatin extraction-Analysis of Variance (ANOVA) for Response Surface Quadratic Model

Source of variation	Degrees of Freedom	Gel Strength		Yield	
ROHU					
		Sum of squares	*P* value*	Sum of squares	*P* value*
Regression	14	33154.19	. 001	146.52	.001
Linear	4	20904.67	. 002	108.17	.002
Square	4	8848.62	. 01	35.04	.01
Interaction	6	2335.06	.080	5.49	0.260
Residue Error	15	2510.11	-	2.81	-
Lack of Fit	10	3334.31	0.060	0.79	0.12
Pure error	5	1175.8	-	0.84	-
Total	29	34598.46	-	151.51	-
R^2	-	95.82%	-	96.71%	-
R^2_{adj}	-	92.90%	-	90.09%	-
COMMON CARP					
Regression	14	31484.16	0.001	140.52	.001
Linear	4	25383.54	0.001	110.11	.001
Squape	4	4096.05	0.04	26.20	.02
Interaction	6	2199.41	0.07	5.19	0.04
Residue Error	15	1267.44	-	2.51	-
Lack of Fit	10	3081.06	0. 08	0.66	0.23
Pure error	5	1186.38	-	0.83	-
Total	29	32946.44	-	144.01	-
R^2	-	95.56%	-	97.58%	-
R^2_{adj}	-	90.92%	-	95.09%	-
GRASS CARP					
Regression	14	46792.67	0.001	56.91	0.001
Linear	4	34582.96	0.002	50.12	0.001
Square	4	8676.71	0.04	4.77	001
Interaction	6	2403.80	0.08	1.07	0.07
Residue Error	15	1701.98	-	1.47	
Lack of Fit	10	3470.54	0.23	0.43	0.32
Pure error	5	1231.44	-	0.64	-
Total	29	47365.45	-	57.43	-
R^2	-	98.79%	-	99.09%	-
R^2_{adj}	-	95.86%	-	98.43%	-

* Significant for $P < 0.05$.

below the range prescribed for Type A (pH 6.0 - 9.5) and Type B Gelatins (pH 4.7 – 5.6). This may be due to the pretreatment with alkali and acid during the extraction process. Choi and Regenstein (2000) observed that the gel strength of the fish and pork gelatins were below pH 4 and slightly above pH 8 respectively. The melting points also showed similar dependencies in relation to pH. Cole (2000) reported that for Type B gelatin, the viscosity is minimum and the gel strength is maximum at pH 5 and hence it is advantageous to manufacture gelatin at this pH. The pH reported for gelatin from the skin of red tilapia was 3.05 and black tilapia 3.91 (Jamilah and Harvinder, 2002).

Table 12. Carp skin gelatin extracion-Optimization Parameters in the Response Optimizer

Name	Goal	Limit		Weight		Importance
		Lower	Upper	Lower	Upper	
			Rohu			
NaOH	minimize	0.1	0.2	1	1	3
H_2SO_4	minimize	0.1	0.2	1	1	3
Pretreatment Time	is in range	45	55	1	1	3
Ext. Temp.	is in range	45	55	1	1	3
Gel Strength	maximize	119.14	221.44	1	1	3
Yield	maximize	6.1	16.88	1	1	3
			Common Carp			
NaOH	minimize	0.1	0.2	1	1	3
H_2SO_4	minimize	0.1	0.2	1	1	3
Pretreatment Time	is in range	40	60	1	1	3
Ext. Temp.	is in range	40	60	1	1	3
Gel Strength	maximize	98.06	211.11	1	1	3
Yield	maximize	7.51	15.42	1	1	3
			Grass Carp			
NaOH	minimize	0.1	0.2	1	1	3
H_2SO_4	minimize	0.1	0.2	1	1	3
Pretreatment Time	is in range	40	60	1	1	3
Ext. Temp.	is in range	40	60	1	1	3
Gel Strength	maximize	98.64	259.91	1	1	3
Yield	maximize	7.22	13.14	1	1	3

Table 13. Carp skin gelatin extraction-Optimization Solutions Obtained Using the Response Optimizer

Number	NaOH	H_2SO_4	Pretreatment Time (min.)	Ext. Temp. (^{0} C)	Gel Strength (B)	Yield (%)	Composite Desirability
Rohu							
1	0.10	0.10	51.35	49.33	189.12	13.06	0.808
Common Carp							
1	0.10	0.10	60.00	57.97	183.01	12.88	0.845
Grass Carp							
1	0.10	0.10	60.00	40.00	230.84	11.43	0.874

The viscosity for the samples was in the range of 5.96 to 7.07. The viscosity was significantly higher ($p<0.05$) for grass carp gelatin followed by rohu and common carp gelatins (Table 15). Viscosity is the second most important commercial property of gelatin after gel strength (Ward & Courts, 1977). Viscosity is partially controlled by molecular weight and molecular size distribution (Sperling, 1985). The viscosities of most of the commercial gelatins have been reported to be in the range of 2.0 to 7.0 cP and up to 13.0cP for specialized ones (Johnston-Banks, 1990). Minimum viscosity for gelatin was observed in the pH range of 6-8 (Stainsby, 1987). Jamilah and Harvinder (2002) reported viscosity values of 3.2cP and 7.12cP for red and black tilapia respectively whereas for channel catfish the optimum value predicted was 3.23 cP (Yang *et al.*,

Table 14. Experimental and Predicted Results of Verification under Optimized Conditions*

	Response	Predicted values	Experimental values
Rohu	Gel strength(Bloom)	189.12	188.6 (3.41)
	Yield (%)	13.06	13.20 (0.90)
Common carp	Gel strength(Bloom)	183.01	181.69 (2.82)
	Yield (%)	12.88	12.10 (0.71)
Grass carp	Gel strength(Bloom)	230.84	228.74 (3.19)
	Yield (%)	11.43	10.62 (0.28)

*Values in brackets are standard deviations of triplicate samples.

Table 15. Proximate composition, pH, Viscosity and Colour of Carp Skin Gelatin*

	Source of skin Gelatin		
	Rohu	Common carp	Grass carp
Moisture (%)	8.10 ± 0.12 ^{a}	8.48 ± 0.11 ^{b}	7.24 ± 0.20 ^{c}
Protein (%)	90.43 ± 0.70	89.71 ± 0.59	91.54 ± 0.75 ^{a}
Lipid (% dwb)	0.57 ± 0.07 ^{a}	0.62 ± 0.06 ^{b}	0.41 ± 0.03 ^{c}
Ash (%)	1.18 ± 0.04 ^{a}	1.11 ± 0.02 ^{b}	1.10 ± 0.07 ^{c}
pH	4.08 ± 0.04	4.05 ± 0.06	4.42 ± 0.04 ^{a}
Yield (%)	12.93 ± 0.55	12.00 ± 0.50	10.57 ± 0.13 ^{a}
Gel strength (Bloom)	188.63 ±2.64 ^{a}	181.31 ±2.08 ^{b}	230.18 ±0.88 ^{c}
Viscosity (cP)	6.06 ± 0.04	5.96 ± 0.12	7.07 ± 0.10 ^{a}
Colour			
L^*	91.89 ± 0.62	90.15 ± 0.64 ^{a}	92.53 ± 0.63
a^*	-0.35 ± 0.02	-0.41 ± 0.03 ^{a}	-0.36 ± 0.02
b^*	2.76 ± 0.21	1.82 ± 0.45 ^{a}	2.70 ± 0.22

*All values were mean ± standard deviation of triplicate analyses. Different superscripts in the same row indicate significant differences ($P < 0.05$).

2007). Gelatin yield was maximum from Rohu followed by Common carp. The yield was significantly low for Grass carp. The yields of gelatin have been reported to vary among the fish species, mainly due to the differences in collagen content, the compositions of skin as well as the skin matrix. Leaching of collagen during the washing treatments of skin could result in the lower yield of gelatin. Insufficient denaturation of soluble collagen during the extraction can also result in lower yield. The acid pretreatment during the extraction removes the non collagen protein as the skin sample swells in the acid solution. The hot water extraction hydrolyses and solublises the gelatin which is then separated by filtration. In this study it is observed that the maximum swelling of the skins during pretreatment with alkali and acid was for rohu and common carp, which indicated a better yield as expected due to the opening of cross links during swelling. Further, a high degree of cross linking via covalent bonds can cause decrease in solubility of collagen and might lead to lower content of extractable gelatin (Foegeding *et al.*, 1996).Variations in gelatin yield have been reported for different species which could be due to the diverse extraction processes (Gomez-Guillen *et al.*, 2002; Jamilah & Harvinder, 2002; Muyonga *et al.*, 2004; Jongjareonrak *et al.*, 2006). The yield observed for the species in this study is comparatively better which offers scope for

commercially viable extraction of gelatin from fish skins.

Gel strengths of gelatins from rohu, common carp and grass carp skins are shown in Table 15. Gel strengths of rohu and common carp skin gelatins were significantly lower than that of grass carp skin gelatin. The gelatins from the skins of rohu, grass carp and common carp have medium gel strengths which are of commercial significance, considering the potential applications in edible food film preparations. Gel strength is one of the most important functional properties of gelatin and fish gelatin typically has less gel strength than mammalian gelatin (Gilsenan & Ross-Murphy, 2000). The gel strengths obtained in this study was in agreement with that reported by Jamilah and Harvinder (2002) for Tilapia (180.76 blooms) and Muyonga *et al.* (2004) for Nile perch (229 g), but lower than that reported by Cho *et al.* (2005) for Yellowfin tuna (426 blooms), Grossman and Bergman (1992) for Tilapia (263 g) and Kasankala *et al.*, (2007) for Grass carp (267 g) which are tropical fish. Lower gel strengths were reported for gelatins from the skins of other tropical species viz., sin croaker (124.94g) and shortfin scad (176.92g) by Cheow *et al.*, (2007). The differences in gel strength among the various species could be explained by differences in manufacturing process used and the intrinsic properties of collagen which varies among fish species. Gudmunsson and Hafsteinsson (1997) suggested that the gel strength may depend on the isoelectric point and may be controlled, to a certain extent, by adjusting the pH. Fish gelatin with lower gel melting temperature had a better release of aroma and offered stronger flavour and useful in product development to control the texture and flavour release during mastication.

Instrumental colour measurements of the freeze dried gelatin powders are shown in Table 15. The gelatins from the skin of rohu, common carp and grass carp had a *snowy* white appearance and were light-textured. The colour of the gelatin depends on the raw material used for the extraction and also whether it is obtained from first stage, second stage or subsequent stages (Ockerman & Hansen, 1999), however, colour does not influence other functional properties. Common carp gelatin showed significantly lower value ($p< 0.05$) for lightness ('L*) than the other two gelatin samples. The a * values for the three gelatin samples showed negative values indicating a shift of colour towards green and it was significantly higher for common carp gelatin. The b* values were positive indicating the degree of yellowness. Common carp gelatin had significantly low b* value than the other samples. However all the gelatin samples appeared to be white in colour on visual observation. This could be a positive attribute, since it is easier to incorporate these gelatins into food system without imparting any colour to the product.

The gelatin from the skin of the three species of freshwater carps showed high yield and medium gel strength which has potential use in food applications. The high viscosity of Carp skin gelatin may be useful for film forming applications. Response Surface Methodology is an ideal tool for optimizing the process parameters for the extraction of gelatin from the skin of Freshwater Carps. The process optimization solutions can be adapted for the industrial extraction of gelatin from carp skins.

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