

AMINO ACID PROFILE OF THE GELATIN EXTRACTED FROM THE SCALES OF CATLA, ROHU, GRASS CARP AND THEIR MIXED TYPE

Muhammad Arif Hossain, Amlan Ganguly¹, S. M. Abdur Rahman¹,
Anwar Hossain, Mahmud Hasan and Mohammad Shamsur Rahman*

*Department of Fisheries, Faculty of Biological Sciences
University of Dhaka, Dhaka-1000, Bangladesh*

Abstract: Gelatin was extracted from the scales of catla (*Catla catla*), rohu (*Labeo rohita*), grass carp (*Ctenopharyngodon idella*), and their mixed type. However, amino acid of the gelatin extracted from the scales of catla and rohu was profiled. While grass carp (0.78 ± 0.03) and mixed scales (0.78 ± 0.02) yielded similar level of gelatin, rohu had the lowest recovery (0.61 ± 0.05). Scales of catla had $0.72 \pm 0.06\%$ gelatin. Of the total 14 amino acids, the highest level of glutamic acid (6.58%) was found in the gelatin extracted from the scales of catla with the lowest level of leucine (0.76%). Gelatin extracted from rohu had the highest percent of glutamic acid (6.14) with lowest percent of leucine (0.59). Of the 10 EAAs, 8 were found in the gelatin extracted from the scales of catla and rohu. While the highest per cent of lysine (4.15) was found in the gelatin extracted from the scales of rohu, the lowest per cent (0.59) was leucine. Quantification of fish scales from the fish markets of Bangladesh and extraction optimization may increase both yield and efficiency.

Key words: Gelatin, fish scales, amino acids profile

INTRODUCTION

Gelatin is one of the most widely used food ingredients and a valuable protein, which is translucent and solid substance, colorless or slightly yellow, nearly tasteless and odorless (Sakr 1997). Its applications in food industries are very broad including enhancing the elasticity, consistency and stability of food products. In pharmaceutical industry, the manufacture of pharmaceutical capsules, ointments, cosmetics, tablet coatings, and emulsions often involve gelatin as one of the important ingredients. Gelatin also finds application in photography and some specialized industries (Djagny *et al.* 2001). The global demand for gelatin has been increasing over the years. The annual world output of gelatin is nearly 326,000 tons (Ahmad and Benjakul 2011), with pig skin-derived gelatin counting for the highest (approx. 80%) in Europe, followed by cattle hides (15%) and remaining 5% come from pig and cattle bones and fish (GME 2016).

*Author for correspondence: shamsur@du.ac.bd ¹Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh.

Gelatin is a product obtained from collagenous raw materials derived from animal by-products through partial hydrolysis. Properties of the gelatin depend on the source and type of collagen. Usually, gelatin has been produced from skin and bones of bovines and skin of porcines (Ward and Courts 1977). Due to the outbreak of mad cow disease or bovine spongi form encephalopathy (BSE) in 1980 and unacceptability of gelatin produced from bovine and/or porcine sources by Muslims, Jews and Hindus, the search for others gelatin raw materials has been initiated and accelerated. Fish gelatin has been identified as an alternative to the mammalian origin, which is accepted as a food additive by those religious groups (Haug *et al.* 2004).

Processing of fish leads to the generation of a large biomass of fish wastes (e.g., skin, bones and fins), which is mostly discarded (~7.3 million tons/year; Kelleher 2005). As a result, there is a quest for investigating an increased utilization of collagenous fish waste for the production of gelatin (Wasswa *et al.* 2007).

Pertinent reports are available on the production of fish gelatin and extraction procedures and characterization of the properties of fish gelatin (Norland 1990, Grossman and Bergman 1992, Liu *et al.* 2008, Kwak *et al.* 2009). Gelatin extraction from alternative sources, especially fish processing waste including skin, bones and/or scales, have received increasing attention (Jongjareonrak *et al.* 2006). Skins and bones from many fish species have been investigated as raw material for gelatin production (Cheow *et al.* 2007). However, current production of fish gelatin is only 1% of the annual world gelatin production (Arnesen and Gildberg 2006).

In Bangladesh, the non-conventional fishery by-products, for example, fish dry scales in the fish markets of Dhaka metropolis has been estimated to be nearly 390 mt found in 40 fish markets that could be increased by nine-folds (Sarker *et al.* 2009). However, assessment of the amino acids from fish gelatin in Bangladesh has never been reported. Therefore, this study for the first time assayed the amino acid composition of gelatin extracted from the scales of catla, rohu, grass carp and their mixed type in Bangladesh.

The objectives of this study were to extract gelatin from fish scales (rohu, catla, grass carp and mixed type fish scales) and to evaluate the gelatin by amino acids profiling.

MATERIAL AND METHODS

Scales of rohu, catla and grass carp were drawn from Gabtoly Fish Market, Dhaka. After harvest, the fish scales were washed by rinsing with water to remove superfluous material and packed in zip lock plastic bag (1 kg/bag). Bags

of the fish scales were stored at $-20 \pm 2^\circ\text{C}$ with a maximum storage of less than 2 months until further analysis. Analyses were done in the Aquatic Laboratory of the Department of Fisheries, and Chemical Biology and DNA Research Lab., Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka.

Gelatin extraction: Fish scales were treated with 2 volumes (w/v) of alkali solution (0.5 - 0.9% NaOH) at room temperature (30°C) for 1 - 5 hours to remove the non-collagen protein and subcutaneous tissue after they were swollen. After the alkali treatment, the scales were neutralized by washing under running tap water until they had a pH of about 7. The scales were then subjected to a final wash with distilled water to remove any residual matter. Gelatin was extracted in distilled water at control temperatures within the range of $70 - 90^\circ\text{C}$ for 1 - 5 hours (Table 1). The ratio used was double amount of distilled water against weight of wet scales. The coarse solids were filtered out with filter cloth and this was followed by vacuum-filtering with a Whatman No. 1 filter paper. The filtered solution was evaporated by holding into a heating plate and finally restored at $4 - 5^\circ\text{C}$ until brittle sheets were formed.

Table 1. Parameters optimized to extract the gelatin from fish scales

Fish scale samples	Scales weight (g)	Conc. of NaOH (w/v) (%)	Treatment time (hr)	Extraction temp. ($^\circ\text{C}$)	Extraction time (hr)
Rohu 1	86	0.5	2	75	2.5
Rohu 2	165	0.5	3	81	2.5
Rohu 3	80	0.7	1.5	82	2
Catla 1	140	0.5	3	80	2.5
Catla 2	105	0.6	2	82	2
Catla 3	160	0.6	3	85	2
Grass carp 1	190	0.9	3	78	2.5
Grass carp 2	250	0.8	2.5	84	3
Grass carp 3	180	0.9	1.5	86	2.5
Mixed type 1	200	0.8	3	80	2.5
Mixed type 2	150	0.7	2	81	2
Mixed type 3	180	0.6	2.5	83	3

Calculation of yield of extracted gelatin: The yield was calculated as follows:
Yield (%) dried gelatin (g) \times 100/weight of wet scales used (g).

Determination of amino acid composition: Amino acid composition of the gelatin was determined by using an amino acid analyzer which could only determine 14 amino acids. Some 0.5 g dried gelatin was pasted with 50 ml 6N HCl by mortar pestle, filter and filtrate was hydrolyzed 22 - 24 hours in a hydrolyser apparatus. After hydrolyzing, HCl was removed from filtrate by

evaporating in water bath for 3 - 4 times with distilled water. Final evaporation of the solution was volume up to 25 ml in a volumetric flask by 0.1N HCl which was used to determine amino acids (Anon. 1993).

Statistical analysis: All data were transformed into square root before statistical analysis. Means were compared by one way ANOVA followed by Tukey's HSD post hoc for multiple comparisons. Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) v. 20.0 for windows (SPSS, SAS Institute Inc. Cary, USA). The data were presented as mean \pm standard error of the means (SEM) with the level of significance at $p < 0.05$.

RESULTS AND DISCUSSION

Yield of gelatins (%) obtained from different fish: While grass carp scales yielded the highest per cent of gelatin (0.78 ± 0.03), rohu had the lowest recovery (0.61 ± 0.05 ; Fig. 1). Scales of catla had $0.72 \pm 0.06\%$ gelatin. However, mixed types of fish scales yielded similar level of gelatin ($0.78 \pm 0.02\%$) as grass carp.

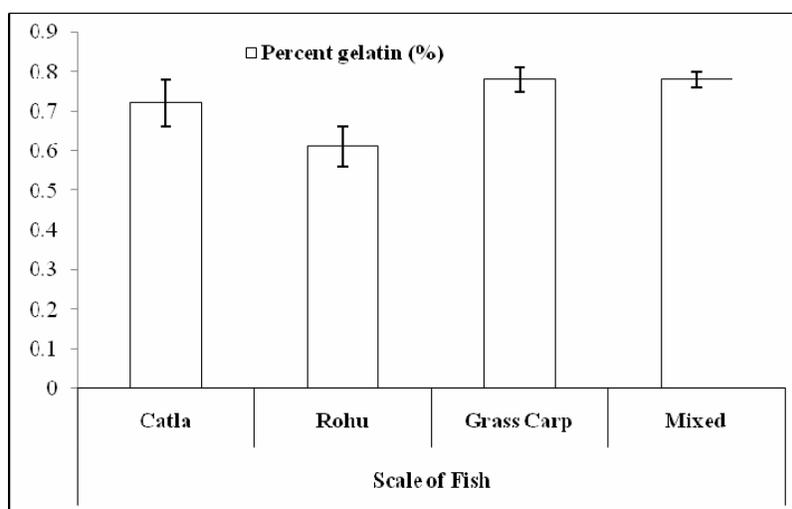


Fig. 1. Gelatin (%) extracted from the scales of catla, rohu, grass carp and their mixed type of scales. Bars (mean \pm 1 SEM) with no letters denote no significant difference between fish ($p < 0.05$).

The difference in the gelatin yield could be due to the difference in the characteristics of the scale of the fish. The yield of gelatin is very low comparable with others. Cheow *et al.* (2007) reported that the yields of gelatin obtained from the skin of sin croaker and short fin scad were 14.3 and 7.25%, respectively. The yield of gelatin obtained from lizard fish scale was 10.6% (Wangtueai and Noomhorm 2009), 19.3% from tilapia skin (Zeng *et al.* 2010), 18.49% from scales

of farmed sea bass *Dicentrarchus labrax* (Dinçer *et al.* 2015). However, the lower yield in the present study could be due to poor extraction methods.

Amino acid composition (g/100 g): Of 14 amino acids, highest per cent of glutamic acid (6.58%) was found in the gelatin extracted from the scales of catla and the lowest per cent was leucine (0.76%; Table 2). The per cent methionine and lysine found were 0.79 and 4.36, respectively. Arginine was also present at 1.36% in the gelatin extracted from the scales of catla. On the other hand, highest per cent of glutamic acid (6.14) was found in the gelatin extracted from the scales of rohu and the lowest per cent was leucine (0.59). The percent methionine and lysine found were 0.85 and 4.15, respectively. Arginine was also present at 1.33% in the gelatin extracted from the scales of rohu.

Table 2. Essential and non-essential amino acid composition of extracted gelatin from rohu and catla fish scales

Sl. No.	Protein and amino acids	Results	
		Catla fish scale gelatin (%)	Rohu fish scale gelatin (%)
	Protein	62.89	58.45
	Essential amino acids		
1	Arginine	1.36	1.33
2	Histidine	2.00	2.10
3	Isoleucine	3.18	2.93
4	Leucine	0.76	0.59
5	Lysine	4.36	4.15
6	Methionine	0.79	0.85
7	Threonine	1.08	0.96
8	Valine	2.30	2.19
	Non-essential amino acids		
9	Aspartic acid	4.44	3.90
10	Serine	2.41	2.05
11	Glutamic acid	6.58	6.14
12	Glycine	4.74	4.33
13	Alanine	3.24	2.95
14	Tyrosine	2.40	1.84

Essential amino acid (EAA) composition (g/100 g): Of 10 EAAs, 8 were found in the two samples (Table 2). Among them, the highest per cent of lysine (4.36) was found in the gelatin extracted from the scales of catla and the lowest per cent was leucine (0.76). While the highest per cent of lysine (4.15) was found in the gelatin extracted from the scales of rohu, the lowest per cent (0.59) was leucine.

The composition of amino acids is of particular importance regarding both gelatin gel strength and melting point (Badii and Howell 2005). The gelatins

extracted in this study contained 14 amino acids, with glutamic acid being the most predominant one. The imino acid (proline and hydroxy proline) and glycine contents of the lizardfish scales gelatin were 20.4 and 18.3%, respectively (Wangtueai and Noomhorm 2009). The content of imino acid and glycine are

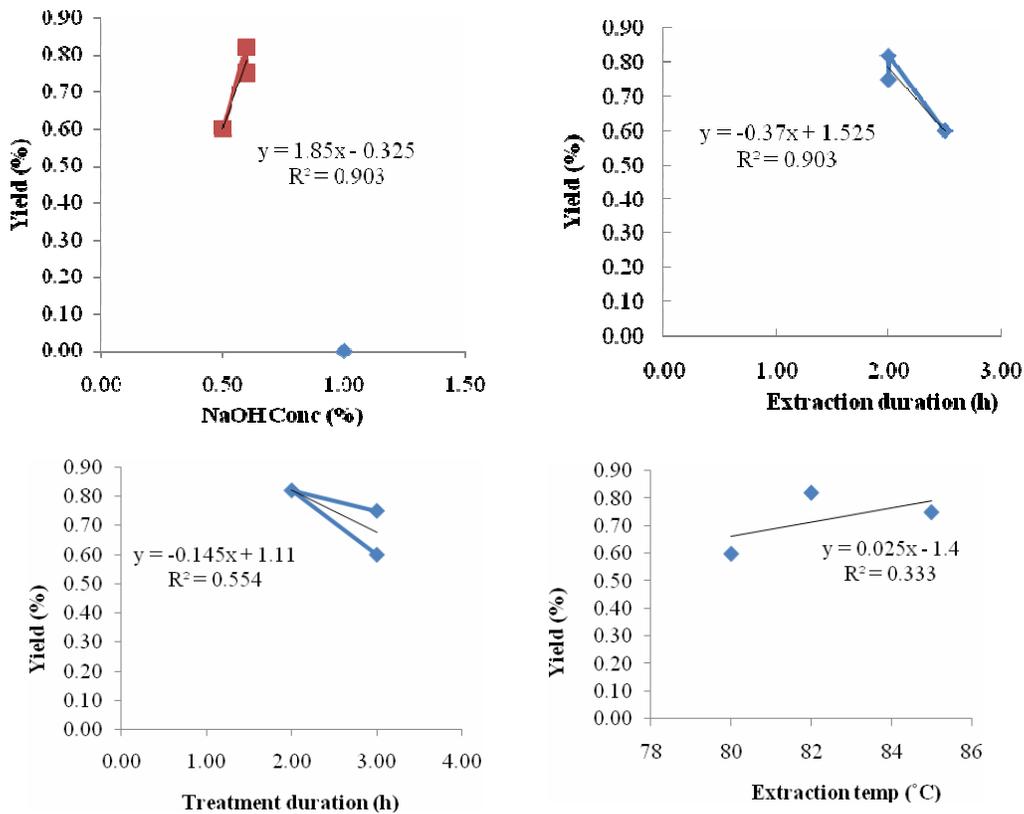


Fig. 2. Relationship between yield (%) of gelatin and the concentration of NaOH (%), extraction duration (hr), extraction temperature (°C) and treatment duration (hr) from the scales of catla.

important for gel strength. A low content of imino acids indicates poor gelling power (Ward and Courts 1977), but imino acid neither proline nor hydroxyproline was found in this study. Glycine content of sea bass scale gelatin (24.4%) was reported by Dinçer *et al.* (2015) which is nearly the same as in big eye snapper skin gelatin as reported by Benjakul *et al.* (2009) but lower than in sole, megrim, and cod gelatin (Nikoo *et al.* 2011). Almost all types of essential amino acids (EAA) were found except phenylalanine and tryptophan with lysine being the most predominant one. All types of amino acids including essential amino acids were found higher percentage in catla sample than rohu except

methionine and histidine. This could be due to the higher protein percent in catla (62.89) than in rohu (58.45). Zeng *et al.* (2010) found 88.5% protein from tilapia skin. Matmaroh *et al.* (2011) reported 43.43% protein in common carp scale and 34.46% protein in golden goatfish. Dincer *et al.* (2015) reported 96% protein in farmed sea bass scale. The changes in protein percentage values depend on the species.

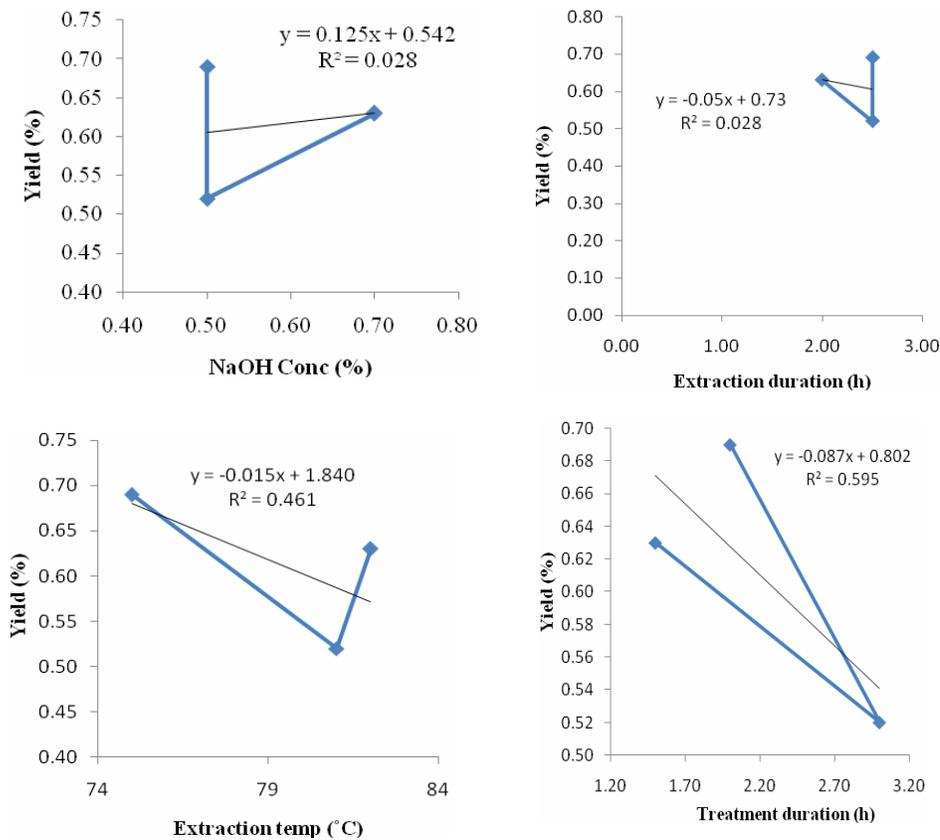


Fig. 3. Relationship between yield (%) of gelatin and the concentration of NaOH (%), extraction duration (hr), extraction temperature (°C) and treatment duration (hr) from the scales of rohu.

Relationship between gelatin yield (%) with the concentration (%) of NaOH, extraction duration (hr), extraction temperature (°C) and treatment duration (hr): In catla, the yield (%) of gelatin had 90.3% positive correlation with the concentration of NaOH (%) and extraction duration (hr) each. The regression equation showed that if the concentration of NaOH is increased and extraction duration is reduced the yield will be increased. The yield (%) of gelatin had 55.4

and 33.3% positive correlation with the treatment duration (hr) and extraction temperature ($^{\circ}\text{C}$), respectively (Fig. 2).

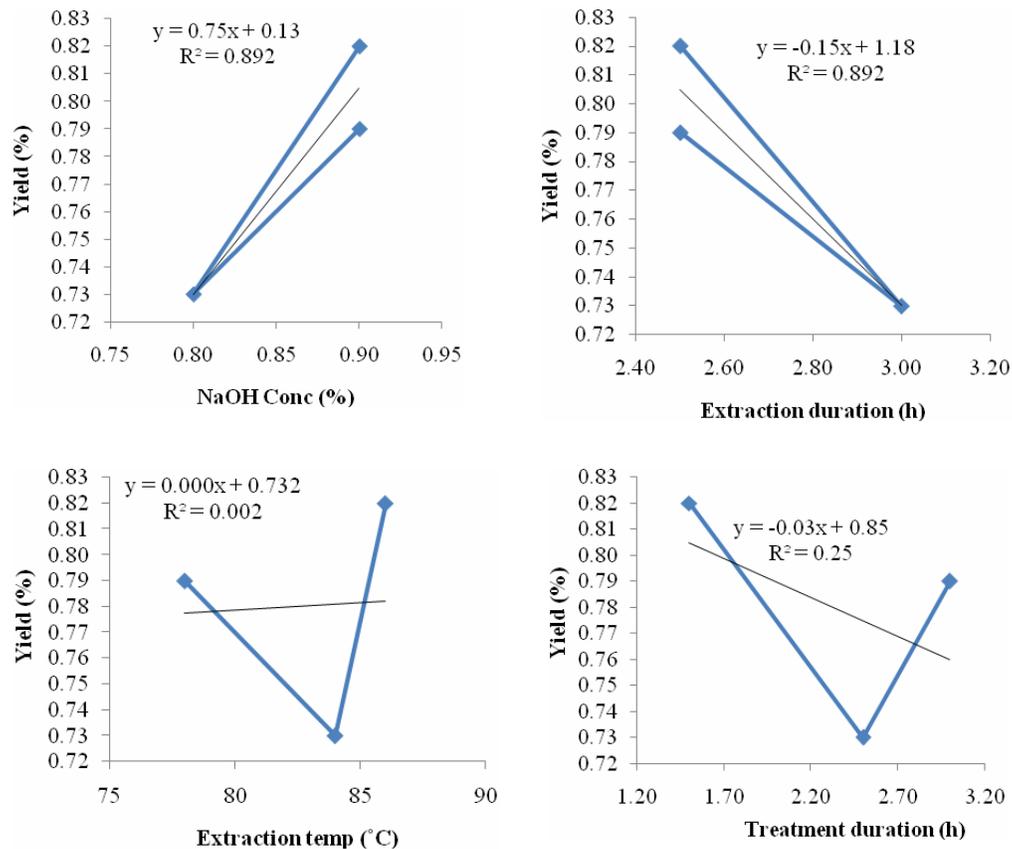


Fig. 4. Relationship between yield (%) of gelatin and the concentration of NaOH (%), extraction duration (hr), extraction temperature ($^{\circ}\text{C}$) and treatment duration (hr) from the scales of grass carp.

In rohu the yield (%) of gelatin had 59.59 and 46.12% positive correlation with the treatment duration (hr) and extraction temperature ($^{\circ}\text{C}$), respectively (Fig. 3).

In grass carp the yield (%) of gelatin had 89.2% positive correlation with the concentration of NaOH (%) and extraction duration (hr). The regression equation showed that if the concentration of NaOH is increased and extraction duration is reduced the yield will be increased (Fig. 4).

In mixed sample the yield (%) of gelatin had 96.4% positive correlation with the treatment duration (hr). The regression equation showed that if the treatment duration is increased the yield will be reduced (Fig. 5).

The effects of four independent variables like concentration of NaOH (%), treatment time (hr), extraction temperature (°C) and extraction time (hr) were determined. In catla, the yield of gelatin was proportionally related with NaOH concentration (%) and extraction temperature (°C) but inversely related with extraction duration (hr) and treatment duration (hr). In case of rohu, the yield of gelatin was only proportionate with NaOH concentration (%) and inversely

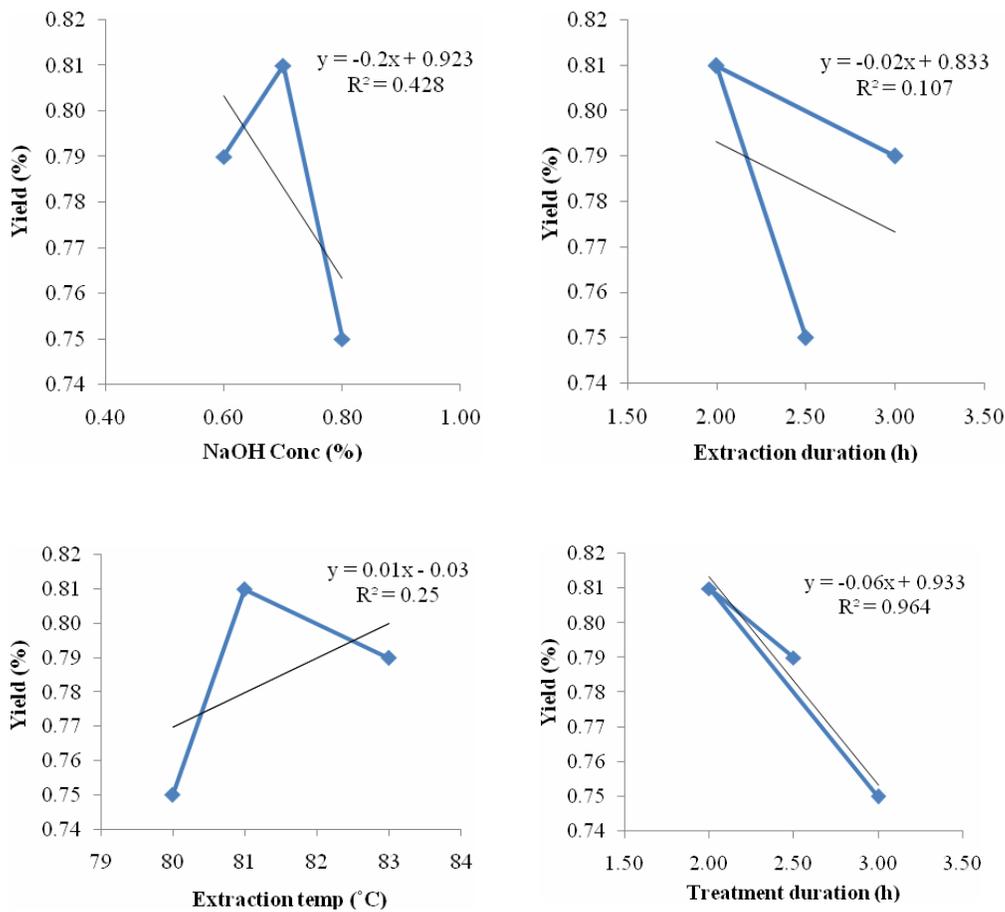


Fig. 5. Relationship between yield (%) of gelatin and the concentration of NaOH (%), extraction duration (hr), extraction temperature (°C) and treatment duration (hr) from the scales of mixed types of scales.

related with extraction temperature (°C), extraction duration (h) and treatment duration (hr). The yield (%) of gelatin in grass carp had a proportional relation with NaOH concentration (%) and extraction temperature (°C) but inversely related extraction duration (hr) and treatment duration (hr). However, in mixed types of scales the yield (%) of gelatin had a proportional relation only with

extraction temperature (°C) but inversely related with NaOH concentration (%), extraction duration (hr) and treatment duration (hr).

Amino acid composition of the gelatin found in catla indicates its suitability as a raw material of protein from fish gelatin compared to rohu. However, scales of grass carp and mixed type could also be used as the source of good quality fish gelatin. Only single study was found on the optimization and characterization of gelatin from lizard fish scale (Wangtueai and Noomhorm 2009). Therefore, considering the above mentioned points, the following recommendations are being made for further studies: (1) Need to quantify the total harvest of dry scales from all fish markets in Bangladesh and (2) Need to develop a standard technique of extraction of gelatin from fish scales.

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