YIELD OF GELATIN FROM CATLA, ROHU, GRASS CARP AND MIXED SCALES AND THEIR AMINO ACID COMPOSITION

A thesis submitted to the Department of Fisheries, University of Dhaka in partial fulfillment of the requirements for the degree of Master of Science in Fisheries

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DEDICATED

TO

MY BELOVED PARENTS

CERTIFICATE

We certify that this thesis entitled "**Yield of Gelatin from Catla, Rohu, Grass Carp and Mixed Scales and their Amino Acid Composition"** is submitted by **Muhammad Arif Hossain**, Roll No.: Curzon-703, Session: 2014-15, Registration No. 2010-413-009/2010-11, has been carried out under our supervision.

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Abstract

Gelatin is mostly used as food ingredients. Its applications in food industries varies from enhancer as elasticity, consistency and stability of food products. Gelatin is also used as a stabilizer, particularly in dairy 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.

Gelatin is obtained from collagenous raw materials mostly from skin and bones of bovines and skin of porcines those are not "Halal". Therefore, there is a need to extract gelatin from 'Halal' sources like fish scales and skins which may be collected from fish market and fish processing industries. This study extracted gelatin from the scales of catla, rohu, grass carp and mixed type. Thereafter, amino acid profiling of the gelatin extracted from the scales of catla and rohu was done.

Grass carp (0.78 ± 0.03) and mixed types of scales (0.78 ± 0.02) yielded similar level of gelatin percent. However, rohu had the lowest recovery of percent gelatin (0.61 ± 0.05). Scales of catla had 0.72 ± 0.06% gelatin.

Of the total 14 amino acids, highest percent of Glutamic acid (6.58 %) was found in the gelatin extracted from the scales of catla with lowest percent of Leucine (0.76 %). The percent 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.

Gelatin extracted from rohu had highest percent of Glutamic acid (6.14 %) with lowest percent of 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.

Of the 10 EAAs, 8 were found in the two of gelatin extracted from the scales of catla and rohu. Among them highest percent Lysine (4.36%) was found in the gelatin extracted from the scales of catla. While the highest percent of Lysine (4.15 %) was found in the gelatin extracted from the scales of rohu, the lowest percent (0.59%) was Leucine.

Quantification of fish scales from the fish markets of Bangladesh and extraction optimization may enhance both yield and efficiency.

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List of Symbols and Abbreviations

kg	Kilogram
°C	Degree Celsius
%	Percentage
h	Hour
g	Gram
w/v	Weight/Volume
mL	Milliliter
N	Normality
et al.	and others
cm	Centimeter
BW	Body Weight
mt	Metric Ton

Chapter 1

Introduction

1.1 Background

Gelatin is one of the most widely used food ingredients. Its applications in food industries are very broad including enhancing the elasticity, consistency and stability of food products. Gelatin is also used as a stabilizer, particularly in dairy products (Gimenez *et al.*, 2005). In the 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.*, 2011).

Gelatin is a product obtained from collagenous raw materials. Hence, the properties of gelatin depend on the source and type of collagen. Normally gelatin has been produced from skin and bones of bovines and skin of porcines (Hinterwaldner, 1977; Ward and Courts, 1977). Due to the outbreak of mad cow disease or Bovine Spongiform Encephalopathy (BSE) in 1980 as well as the unacceptability of gelatin produced from bovine and/or porcine sources for Muslims, Jews and Hindus, the search for others gelatin raw materials has been initiated and accelerated. An alternative to the mammalian gelatin, which is accepted as a food additive by those religious groups, is fish gelatin (Haug, Draget and Smidsrød, 2004).

Gelatin is a valuable protein derived from animal by-products obtained through partial hydrolysis of collagen originated from cartilages, bones, tendons and skins of animals. It is a translucent brittle solid substance, colorless or slightly yellow, nearly tasteless and odorless (Sakr, 1997). Today, gelatin is usually available in granular powder form, although in Europe countries, sheet gelatin is still available (Sakr, 1997). Most commercial gelatin is currently sourced from beef bone, hide, pig skin and, more recently, pig bone. It was reported that 41% of the gelatin produced in the world is sourced from pig skin, 28.5% from bovine hides and 29.5% from bovine bones (Hayatudin, 2005). In recent times, the concern and fear of BSE or “mad cow disease” has affected the gelatin market and has shifted the market towards porcine gelatin.

The global demand for gelatin has been increasing over the years. Recent reports indicate that the annual world output of gelatin is nearly 326,000 tons, with pig skin-derived gelatin accounting for the highest (46%) output, followed by bovine hides (29.4%), bones (23.1%), and other sources (1.5%) (GME, 2008). However, although gelatin has such a wide range of useful applications, pessimism and strong concerns still persist among consumers with regard to its usage (Asher, 1999). This is mainly due to religious sentiments (both Judaism and Islam forbid the consumption of any pork-related products, while Hindus do not consume cow-related products) as well as the enhanced and stricter adherence to vegetarianism throughout the world. In addition, there is increasing concern among researchers about whether animal tissue-derived collagens and gelatins are capable of transmitting pathogenic vectors such as prions (Wilesmith, Ryan and Atkinson, 1991).

Fish catch is mainly used for human consumption and other minor uses, such as meal production and bait. Fish used as human food accounts for 78% of the total fish catch in developed and developing countries, leaving about 21% for non-food uses (Vannuccini, 2004). Processing leads to the generation of a large biomass of fish waste (e.g., skin, bones, and fins), which is generally discarded (~7.3 million tons/year) (Kelleher, 2005). Consequently, research has been initiated to investigate an increased utilization of collagenous fish waste for the production of gelatin (Gilsenan and Ross Murphy, 2000; Holzer, 1996; Nagai and Suzuki, 2000; Wasswa, Tang and Gu, 2007).

Production of fish gelatin is actually not new as it has been produced since 1960 by acid extraction, although most of it has been used for industrial applications (Norland, 1990). Detailed extraction procedures and characterization of the properties of fish gelatin were described by Grossman and Bergman (1992) in a United States patent. Since then, multiple research groups have further investigated the various aspects of fish gelatin. Gelatin has been extracted from skins and bones of various cold-water (e.g., cod, hake, Alaska pollock, and salmon) and warm-water (e.g., tuna, catfish, tilapia, Nile perch, shark, and megrim) fish.

It is well established that temperature characteristics of collagen and gelatin from fish skin reflect the fish habitat temperature (Andreva, 1971). The composition of amino acids is of particular importance regarding both gelatin gel strength and melting point. Due to the rigidity of their R-groups in the amino acids provide rigidity to triple helix structures both in intact collagen and gelatin gels. Apparently, high contents of hydrophobic amino acids have a similar effect, although less prominent (Badii and Howell, 2005). Gelatin is a product of partial hydrolysis of collagen and is usually extracted from porcine or bovine bones and skins. In the past 15 years, considerable effort has been expended on studying gelatin extraction from different fish species (Kołodziejska *et al.,* 2004, 2008; Zhou and Regenstein, 2005; Zhou *et al.,* 2006; Cho *et al.,* 2005; Liu *et al.,* 2008; Muyonga *et al.,* 2004; Aewsiri *et al.,* 2008; Kwak *et al.,* 2009). However, the production of fish gelatin is still in its infancy, contributing only about 1% of the annual world gelatin production (Arnesen and Gildberg, 2006). There are some limiting factors such as insufficient availability of raw materials, inferior rheological properties, prices. These problems have hampered the large-scale development of fish gelatin industry.

One of the most important characteristics of gelatin is that it has a low melting temperature below human body temperature that makes its use very favorable in the food industry (Choi and Regenstein 2000). Another important characteristic of gelatin is that its gel strength is relatively higher than most of the common gelling agents, which are usually carbohydrates obtained from vegetable sources (Badii and Howell 2006). Carbohydrate-based gelling agents have much higher melting temperatures along with less gel strength. A unique disadvantage of gelatin, however, is its challenges with regard to kosher and halal status, since almost half of the world's gelatin production is obtained from pig skin while the rest comes from animals that have not been religiously slaughtered. In addition, vegetarians also have objections to its usage since gelatin is derived from animal hides and bones, although some vegetarians do accept ingredients derived from fish (Choi and Regenstein 2000).

1.2 Dry scale marketing in Bangladesh

The non-conventional fishery by-products like fish dry scales in the fish markets in Dhaka metropolis were identified and quantified by structured survey while the marketing channels were examined through observation (Sarker *et al.*, 2009). Estimated total yield of dry scales found was 390 mt which could be increased by 9-folds. Dry scales are mainly collected by cutters and cutter-cum-sellers. The cutters were found the highest quantity while the cutter-cum-sellers did not. The unwillingness of the cutter-cum-sellers in collecting dry scales could be due to the perception of risk in reducing their social status compared to the cutters. Another reason for not collecting the dry scales by the cutter-cum-sellers could be labor intensiveness. The yield of dry scale per collector per annum was 750 kg. However, 52 (12%) collectors (C/CCS) were found collecting dry scales. Thus total yield in the sampled 40 markets would be 39 mt/y.

1.2.1 Expected Potential Total Yield (kg) and Revenue (tk)

The current estimated yield of dry scales was nearly 39 mt found in 40 fish markets. However, total markets found were 395. Thus the total yield of dry scales estimated would be 390 mt in Dhaka metropolis (Table 1). If all cutters and cutter-cum-sellers would harvest, the total harvest could be 3390 mt which is 869 percent 9-folds higher than the current level.

Table 1: Current and Expected Potential Yield of dry scales of Surveyed Fish Markets

Item	Current yield		Expected potential yield		Increment of the current yield (%)
	Collector	Quantity	Collector	Quantity	
Dry scale	52	39 mt	452	339 mt	869

It is important to note that in 2006, the average market price of dry scales was US$0.29/kg. The value of the estimated potential yield of dry scale in Dhaka metropolis thus would be US$1 million which is 869 per cent increment of the current US$0.11 million per annum (Table 2).

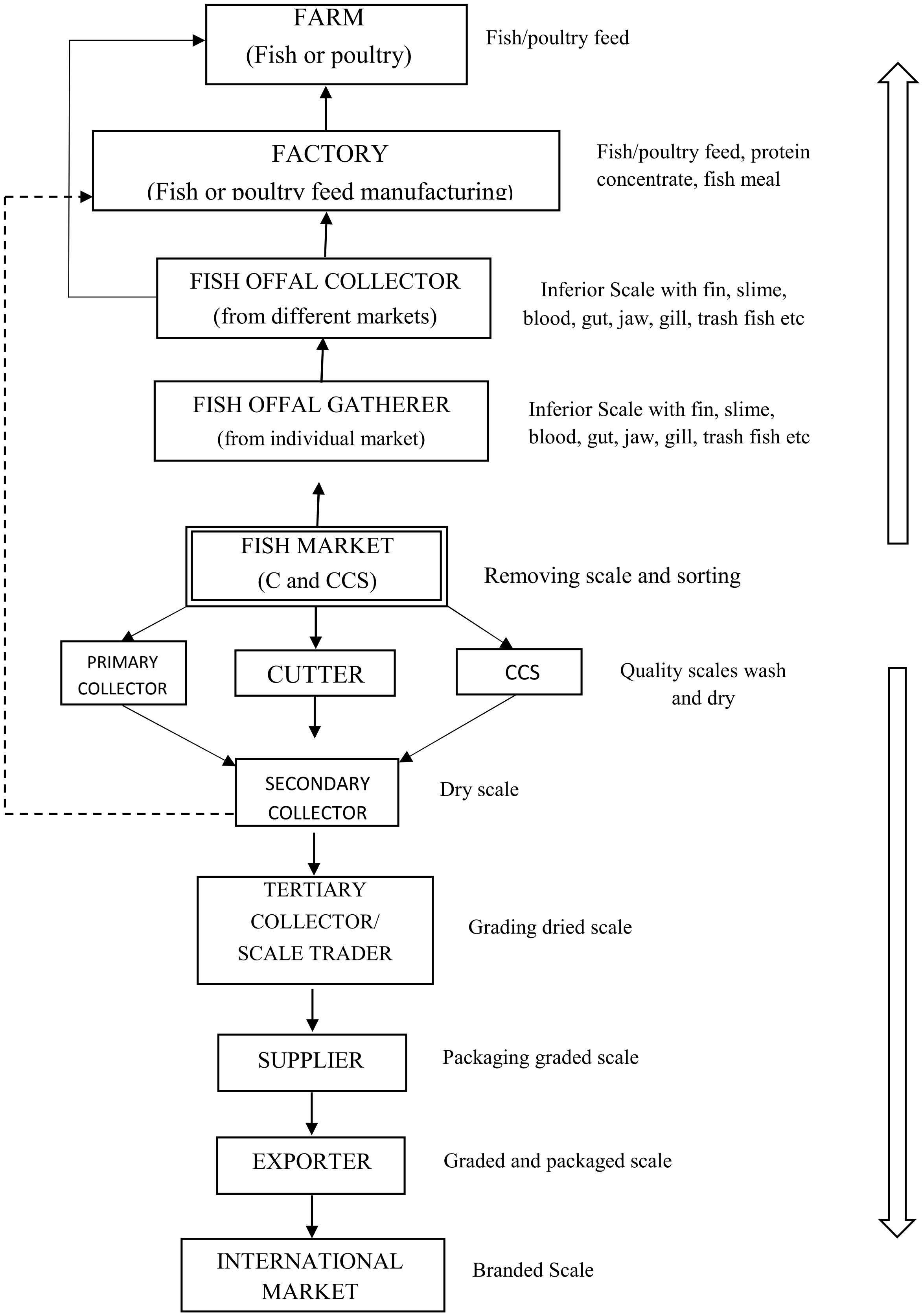
Table 2: Current and Expected Potential Value of dry scales of Surveyed Fish Markets

Item	Current value (US$)	Expected potential value (US$)	Increment of the current value (%)
Dry scale	11340	98571	869

1.2.2 Marketing channels

Marketing channel of dry scale is given Figure 1:

Figure 1: Marketing Channels of Scale (with fish offal) in and Outside of Dhaka Metropolitan City.



Note: Dashed line indicates secondary trading channel. (Sarker *et al.*, 2009).

1.3 Fish biology

1.3.1 Rohu

Rohu (*Labeo rohita*) is the most important among the three Indian major carp species used in carp polyculture systems. This graceful Indo-Gangetic riverine species is the natural inhabitant of the riverine system of northern and central India, and the rivers of Pakistan, Bangladesh and Myanmar. In India, it has been transplanted into almost all riverine systems including the freshwaters of Andaman, where its population has successfully established. The species has also been introduced in many other countries, including Sri Lanka, the former USSR, Japan, China, Philippines, Malaysia, Nepal and some countries of Africa. The traditional culture of this carp goes back hundreds of years in the small ponds of the eastern Indian states.

Plate 1: Rohu *Labeo rohita* (Hamilton, 1822)

In its early life stages rohu prefer zooplankton, mainly composed of rotifers and cladocerans, with phytoplankton forming the emergency food. In the fingerling stage, there is a strong positive selection for all the zooplanktonic organisms and for some smaller phytoplankters like desmids, phytoflagellates and algal spores. On the other hand, adults show a strong positive selection for most of the phytoplankton. In the juvenile and adult stages rohu is essentially an herbivorous column feeder, preferring algae and submerged vegetation. Furthermore, the occurrence of decayed organic matter and sand and mud in its gut suggests its bottom feeding habit. The nibbling type of mouth with soft fringed lips, sharp cutting edges and absence of teeth in the bucco-pharyngeal region helps the fish to feed on soft aquatic vegetation which do not require seizure and crushing. The modified thin and hair-like gill rakers also suggest that the fish feed on minute plankton through sieving water. In ponds, the fry and fingerlings exhibit schooling behavior mainly for feeding; however, this habit is not observed in adults.

Rohu is a eurythermal species and does not thrive at temperatures below 14 °C. It is a fast growing species and attains about 35-45 cm total length and 700-800 g in one year under normal culture conditions. Generally, in polyculture, its growth rate is higher than that of mrigal but lower than catla.

The minimum age at first maturity for both sexes is two years, while complete maturity is reached after four years in males and five years in females. In nature, spawning occurs in the shallow and marginal areas of flooded rivers. The spawning season of rohu generally coincides with the south-west monsoon, extending from April to September. In captivity with proper feeding the species attains maturity towards the end of second year. However, breeding does not take place in such lentic pond environments; thus induced breeding becomes necessary. The fecundity varies from 226 000 to 2 794 000, depending upon fish size and ovary weight; on average it ranges from 200 000-300 000 eggs/kg BW. Rohu is a polygamous fish and also seems to be promiscuous. The optimum temperature for spawning is 22-31 °C.

1.3.2 Catla

Catla (*Catla catla*) is endemic to the riverine system in northern India, Indus plain and adjoining hills of Pakistan, Bangladesh, Nepal and Myanmar, and has been introduced later into almost all riverine systems, reservoirs and tanks all over India. As the species breeds in the riverine ecosystem, its ready seed availability has helped in establishing its aquaculture in the peripheral region of the riverine system in these countries. The natural distribution of catla seems to be governed by temperature dependency rather than latitude and longitude.

Catla is a eurythermal species that grows best at water temperatures between 25-32 °C. The eggs are demersal at first, gradually becoming buoyant. Early-stage larvae remain in surface and sub-surface waters and are strongly photo tactic. The larvae begin to feed three days after hatching, while their yolk sacs persist. As they increase in size, the number of gill rakers and gill filaments also increases, thus assisting them to strain ingested food items.

The fry are planktophagic, feeding mainly on zooplankton such as rotifers and cladocerans. Adults feed only in surface and mid-waters; they also are planktophagous, with a preference for zooplankton, mainly crustaceans, rotifers, insects and protozoa, as well as a considerable share of algal and plant material.

Plate 2: Catla *Catla catla* (Hamilton, 1822)

Catla attains maturity in its second year, performing a spawning migration during the monsoon season towards the upper stretches of rivers, where males and females congregate and breed in shallow marginal areas. The spawning season coincides with the south-west monsoon in north-eastern India and Bangladesh, which lasts between May and August, and in north India and Pakistan from June to September. Its fecundity generally varies from 100 000-200 000/kg BW, depending on fish length and weight. The resultant seed are brought by water flow to the downstream areas where they are caught by seed collectors.

Since a riverine environment is required, natural breeding does not occur within ponds, even though the species attains maturity; thus hormonal induction is required. Among the three Indian major carps, catla is the most difficult to breed as it requires precise environmental conditions for spawning. Under normal conditions catla grows to 1-1.2 kg in the first year, compared to 700-800 g and 600-700 g for rohu and mrigal, respectively. It attends sexual maturity in two years.

1.3.3 Grass carp

Grass carp (*Ctenopharyngodon idella*) is a native Chinese freshwater fish with a broad distribution from the catchment area of the Pearl River in southern China to that of the

Heilongjiang River in northern China. It has been introduced to about 40 other countries and there have been limited reports about the natural populations occurring in those areas; for instance, a natural population exists in the Red River in Vietnam. It inhabits lakes, rivers and reservoirs. It is a basically herbivorous fish that naturally feeds on certain aquatic weeds. However, the fry/larvae feed on zooplankton. Under culture conditions, grass carp can well accept artificial feed such as the by-products from grain processing, vegetable oil extraction meals, and pelleted feeds, in addition to aquatic weeds and terrestrial grasses.

Grass carp normally dwell in mid-lower layer of the water column. Comparatively, it prefers clear water and can move swiftly. It is a semi-migratory fish; the mature brood stock migrate to the upper reaches of major rivers to propagate. Flowing water and changes in water level are essential environmental stimuli for natural spawning. The fish can reach sexual maturity under culture conditions, but cannot spawn naturally.

Plate 2: Grass carp *Ctenopharyngodon idella* (Valenciennes, 1844)

Hormone injection and environmental stimuli, such as flowing water are necessary for induced spawning in tanks. Gras carp grow rapidly and reach a maximum weight of 35 kg in the wild.

1.4 Rationale

Typically gelatin has been produced from skin and bones of bovines and skin of porcine. Fish gelatin can be used as a food additive. Gelatin from fish is a potential substitute for mammalian gelatins (Pranoto *et al.*, 2007). 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, there is no study on the extraction of gelatin from fish skin and scales in Bangladesh. Therefore, this study may be the first endeavor to find out the gelatin extraction method from fish skin and scales and finally evaluation of the quality of gelatin in Bangladesh.

1.5 Problem statement

The processing of fishery products usually leads to enormous amounts of waste in Dhaka city as well as all over Bangladesh every day, which produces a lot of solid waste including bone, skin and scales. But proper use of this wastage is very low. As gelatin has a profound demand for different purposes, so that these wastage like scales can be used for gelatin extraction. If it will do so, it may improve the economic condition of Bangladesh.

1.6 Objectives

The overall objective of my thesis research work is to evaluate the fish scales as a source of extracting food grade gelatin.

The specific objectives of this study are

- to extract gelatin from fish skin and scales (rohu, catla, grass carp and other fish)
- to evaluate gelatin by profiling the amino acids

Chapter 2

Materials and Methods

2.1 Experimental Fish and Sampling

Scales of rohu, catla and grass carp were drawn from Gabtoly Fish Market, Dhaka, Bangladesh. The fish scales were then 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 ± 2°C with a maximum storage of less than 2 months until further analysis.

2.2 Research Location

The works were conducted at the Aquatic Laboratory of the Department of Fisheries in collaboration with Chemical Biology & DNA Research Lab, Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka.

2.3 Gelatin extraction

At first made 0.5–0.9% alkali solution. Then the scales were treated with 2 volumes (w/v) of alkali solution (0.5–0.9% NaOH) at room temperature (30°C) for 1–5 h 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. The extractions were carried out in distilled water at control temperatures within the range of 70–90°C for 1–5 h. 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 putting into a heating plate. Then restored in 4–5 °C until brittle sheets were formed.

Plate 4: Gelatin of rohu scales

Plate 5: Gelatin of grass carp scales

Plate 6: Gelatin of catla scales

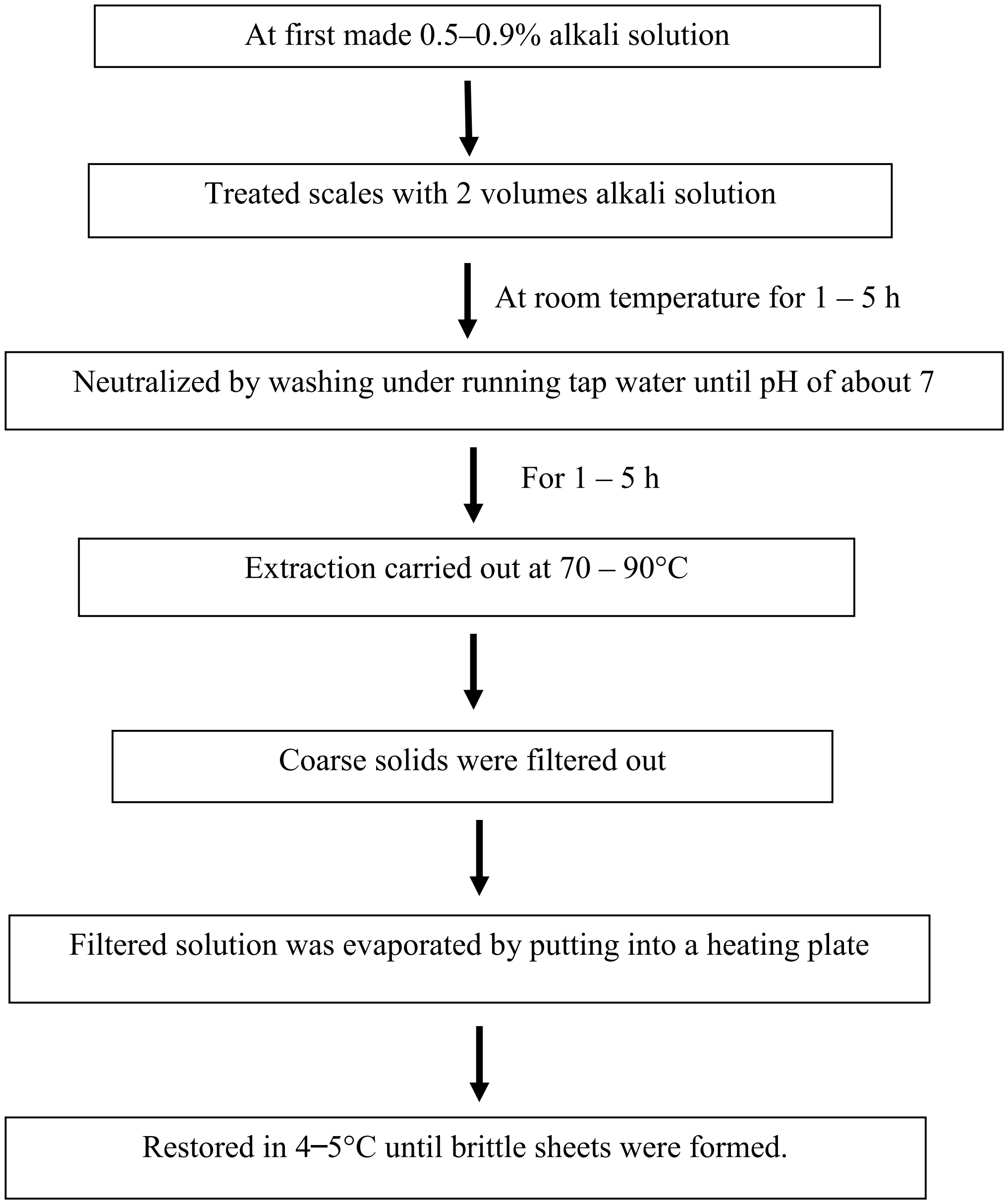


Figure 2: Flowchart of gelatin extraction

Rohu sample – 1

Scale weight	86 g
Concentration of NaOH (w/v)	0.5%
Treatment time	2 h
Extraction temperature	75°C
Extraction time	2.5 h

Rohu sample – 2

Scale weight	165 g
Concentration of NaOH (w/v)	0.5%
Treatment time	3 h
Extraction temperature	81°C
Extraction time	2.5 h

Rohu sample – 3

Scale weight	80 g
Concentration of NaOH (w/v)	0.7%
Treatment time	1.5 h
Extraction temperature	82°C
Extraction time	2 h

Catla sample – 1

Scale weight	140 g
Concentration of NaOH (w/v)	0.5%
Treatment time	3 h
Extraction temperature	80°C
Extraction time	2.5 h

Catla sample – 2

Scale weight	105 g
Concentration of NaOH (w/v)	0.6%
Treatment time	2 h
Extraction temperature	82°C
Extraction time	2 h

Catla sample – 3

Scale weight	160 g
Concentration of NaOH (w/v)	0.6%
Treatment time	3 h
Extraction temperature	85°C
Extraction time	2 h

Grass carp sample – 1

Scale weight	190 g
Concentration of NaOH(w/v)	0.9%
Treatment time	3 h
Extraction temperature	78°C
Extraction time	2.5 h

Grass carp sample – 2

Scale weight	250 g
Concentration of NaOH (w/v)	0.8%
Treatment time	2.5 h
Extraction temperature	84°C
Extraction time	3 h

Grass carp sample – 3

Scale weight	180 g
Concentration of NaOH (w/v)	0.9%
Treatment time	1.5 h
Extraction temperature	86°C
Extraction time	2.5 h

Mixed sample – 1

Scale weight	200 g
Concentration of NaOH (w/v)	0.8%
Treatment time	3 h
Extraction temperature	80°C
Extraction time	2.5 h

Mixed sample – 2

Scale weight	150 g
Concentration of NaOH (w/v)	0.7%
Treatment time	2 h
Extraction temperature	81°C
Extraction time	2 h

Mixed sample – 3

Scale weight	180 g
Concentration of NaOH (w/v)	0.6%
Treatment time	2.5 h
Extraction temperature	83°C
Extraction time	3 h

2.4 Calculation of Yield of extracted gelatin

The yield was calculated as follows:

Yield (%) = dried gelatin (g) × 100/ weight of wet scales used (g).

2.5 Determination of amino acid composition

Amino acid composition of the gelatin was determined by using an amino acid analyzer which could only determine fourteen amino acids. 0.5g dried gelatin was pasted with 50ml 6N HCl by mortar pestle, filter and filtrate was hydrolyzed 22-24h in a hydrolyser apparatus. After hydrolyzing HCl was removed from filtrate by evaporating in water bath for 3-4 times with distill water. Ending of evaporation the solution was volume up to

25ml in a volumetric flask by 0.1N HCl. This stock solution was used for determining amino acids (Anonymous, 1993).

Collagens are major structural proteins of most connective tissues, such as skin, bone and tendons, where they provide structural integrity to the tissues (Bigi *et al.*, 2004). The peculiarity of the amino acid sequence accounts for the characteristic coiled coil structure of the collagen molecule, where three distinct polypeptide chains, each of which is coiled into a left-handed helix are thrown into a right-handed super helix stabilized through inter chain hydrogen bonds and covalent crosslink (Brodsky and Ramshaw, 1997). Thermal denaturation or physical and chemical degradation of collagen involves the breaking of the triple-helix structure into random coils to give gelatin (Bigi *et al.*, 2004). At temperature of about 40ºC, gelatin aqueous solutions are in the sol state and form physical thermo reversible gels on cooling. During gelling, the chains undergo a conformational disorder; order transition and partly regenerate the collagen triple helix structure (Pezron *et al.*, 1991).

According to Stainsby (1987) and Johnston-Banks (1990), amino acid composition plays important role in the physical properties of gelatin. However, the physical properties of the gelatin depends not only on the amino acid composition, but also on the relative content of β - or γ - components and higher molecular weight aggregates, as well as on the presence of lower molecular weight protein fragments. Thus, in addition to the source or species, gelatin properties will also strongly depend on the preservation of raw materials.

2.6 Statistical analysis

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 analyzed to determine the descriptive statistics such as Standard Error of Mean (SEM) and Mean. One way ANOVA was done to test the significance using 5% level of significance.

Chapter 3

Results

3.1 Yield of gelatins (%) obtained from different fish

In comparison, while grass carp scales yielded the highest percent gelatin (0.78 ± 0.03) rohu had the lowest recovery of percent gelatin (0.61 ± 0.05; Figure 3.1). Scales of catla had 0.72 ± 0.06% gelatin. However, mixed types of scales similar level of gelatin (0.78 ± 0.02).

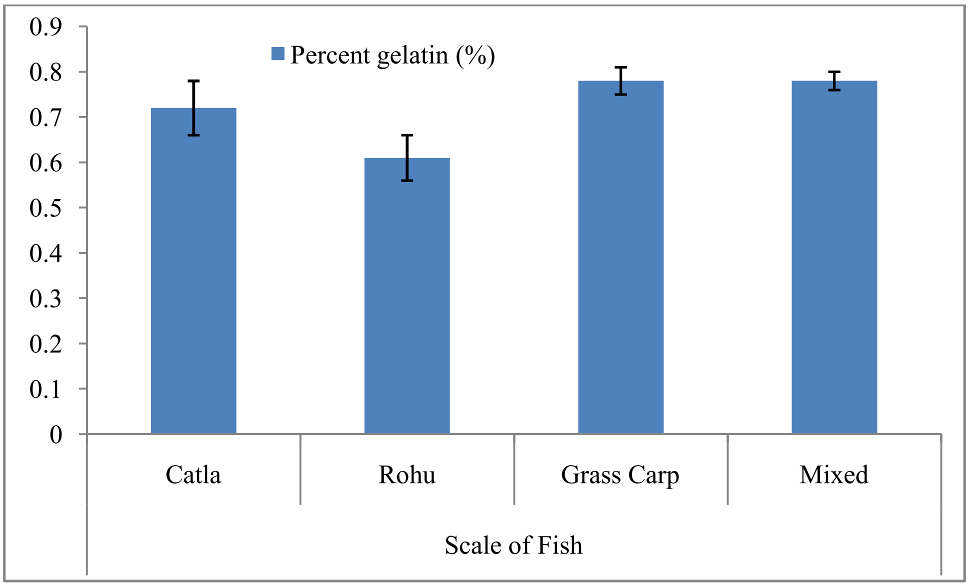


Figure 2: Percent gelatin composition extracted from the scales of catla *Catla catla*, rohu *Labeo rohita*, grass carp *Ctenopharyngodon idella* and mixed type of scales. Bars (mean ± 1 SEM) with no letters denote no significance difference between fish (ANOVA, HSD, P<0.05).

3.2 Amino acid composition (g/100 g)

The amino acid composition of the gelatin from rohu and catla fish scales are shown in the table 3. Of the 14 amino acids, highest percent of Glutamic acid (6.58 %) was found in the gelatin extracted from the scales of catla. Lowest percent of Leucine (0.76 %) was found. The percent 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 percent of Glutamic acid (6.14 %) was found in the gelatin extracted from the scales of rohu. Lowest percent of Leucine (0.59 %) was found. 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 3: Amino acid composition of gelatin from rohu and catla fish scales

SI. No.	**Protein & Amino acids**	**Results (%)**	
		Gelatin (Catla Fish Scale)	**Gelatin (Rui Fish Scale)**
	Protein	**62.89**	**58.45**
1.	Aspartic Acid	**4.44**	**3.90**
2.	Threonine	**1.08**	**0.96**
3.	Serine	**2.41**	**2.05**
4.	Glutamic Acid	**6.58**	**6.14**
5.	Glycine	**4.74**	**4.33**
6.	Alanine	**3.24**	**2.95**
7.	Valine	**2.30**	**2.19**
8.	Methionine	**0.79**	**0.85**
9.	Isoleucine	**3.18**	**2.93**
10.	Leucine	**0.76**	**0.59**
11.	Tyrosine	**2.40**	**1.84**
12.	Histidine	**2.00**	**2.10**
13.	Lysine	**4.36**	**4.15**
14.	Arginine	**1.36**	**1.33**

3.3 Essential Amino Acid (EAA) composition (g/100 g)

The EAA composition of the gelatin from rohu and catla scales is shown in the table 4. Of the 10 EAAs 8 were found in the two samples. Among them highest percent Lysine (4.36%) was found in the gelatin extracted from the scales of catla. However lowest percent of Leucine (0.76 %) was found.

While the highest percent of Lysine (4.15 %) was found in the gelatin extracted from the scales of rohu, the lowest percent (0.59%) was Leucine.

Table 4: Essential amino acid composition of gelatin from rohu and catla scales

SI. No.	Essential amino acids	Gelatin (Catla Fish Scale)	Gelatin (Rohu Fish Scale)
1.	Threonine	1.08	0.96
2.	Valine	2.30	2.19
3.	Methionine	0.79	0.85
4.	Isoleucine	3.18	2.93
5.	Leucine	0.76	0.59
6.	Lysine	4.36	4.15
7.	Histidine	2.00	2.10
8.	Arginine	1.36	1.33

3.4A Relationship between gelatin Yield (%) with the Concentration (%) of NaOH, Extraction duration (h), Extraction temperature (˚ C) and Treatment duration (h) in Catla scales

In catla the yield (%) of gelatin had 90.3% positive correlation with the concentration of NaOH (%) and extraction duration (h). 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 (h) and extraction temperature (˚C) respectively.

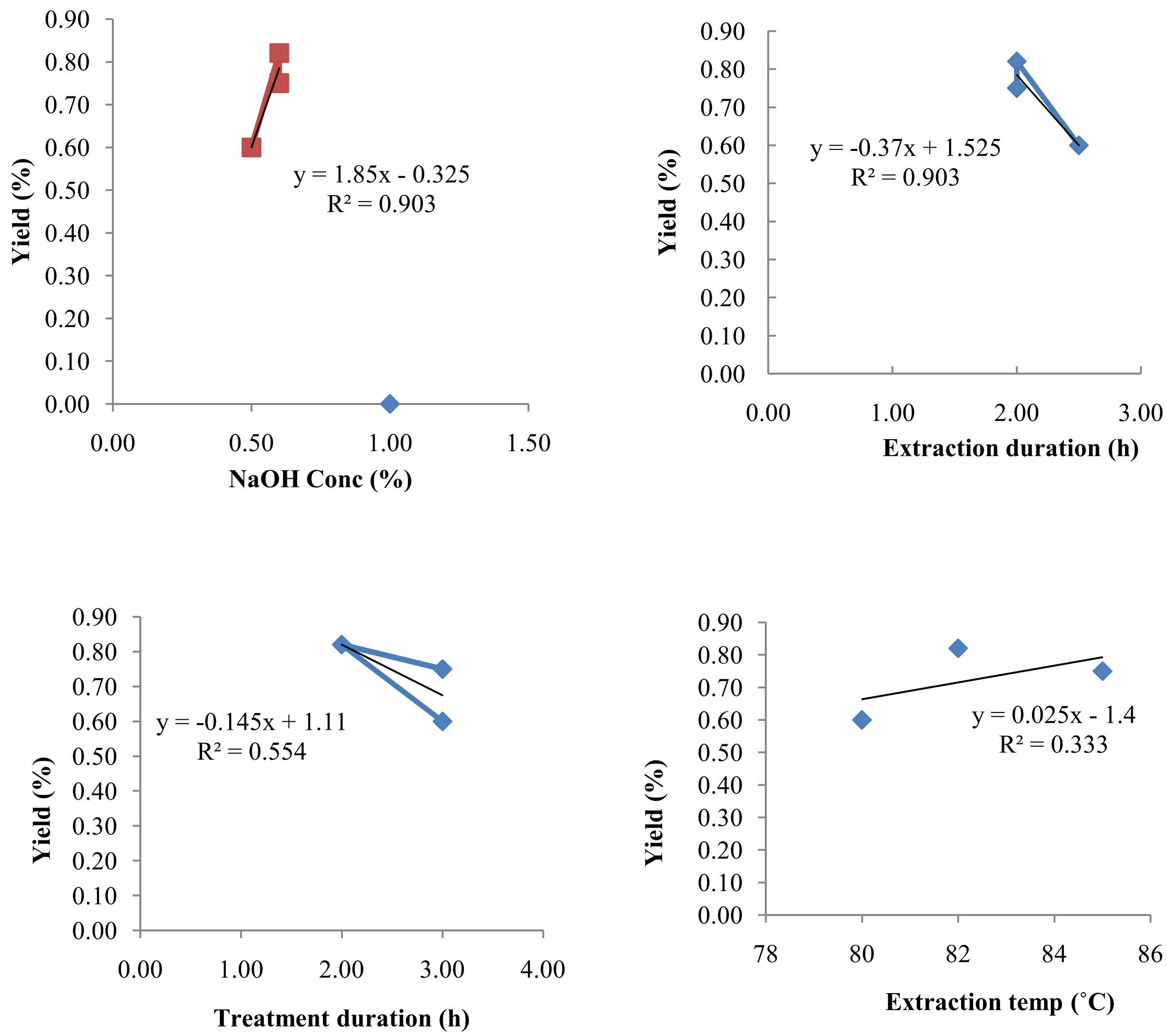


Figure 3: Relationship between yield (%) of gelatin and the concentration of NaOH (%), extraction duration (h), extraction temperature (˚C) and treatment duration (h) from the scales of catla scales.

3.4B Relationship between gelatin Yield (%) with the Concentration (%) of NaOH, Extraction duration (h), Extraction temperature (˚ C) and Treatment duration (h) in Rohu scales.

In rohu the yield (%) of gelatin had 59.5% positive correlation with the treatment duration (h).

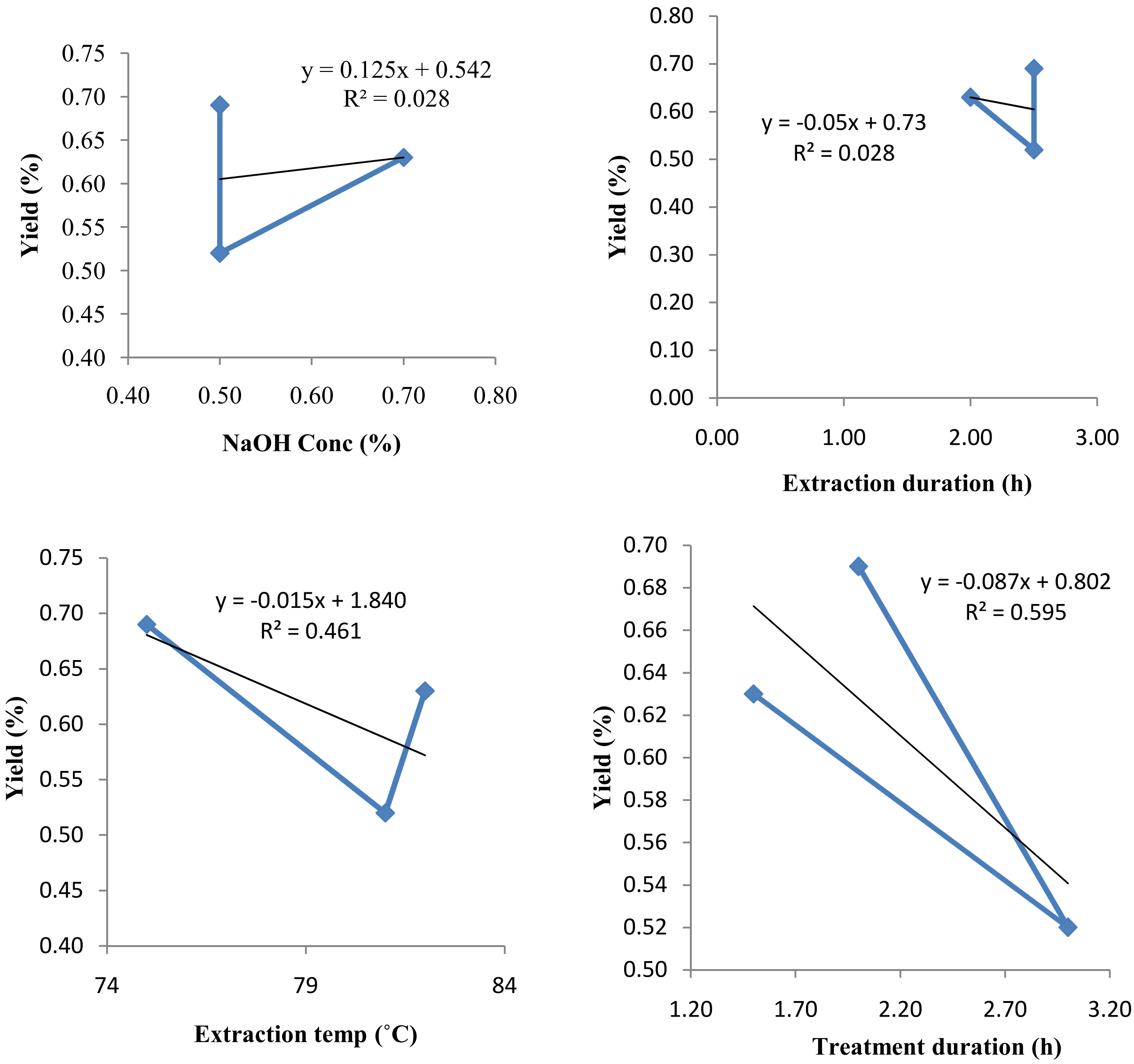


Figure 4: Relationship between yield (%) of gelatin and the concentration of NaOH (%), extraction duration (h), extraction temperature (˚C) and treatment duration (h) from the scales of rohu scales.

3.4C Relationship between gelatin Yield (%) with the Concentration (%) of NaOH, Extraction duration (h), Extraction temperature (° C) and Treatment duration (h) in Grass carp scales.

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

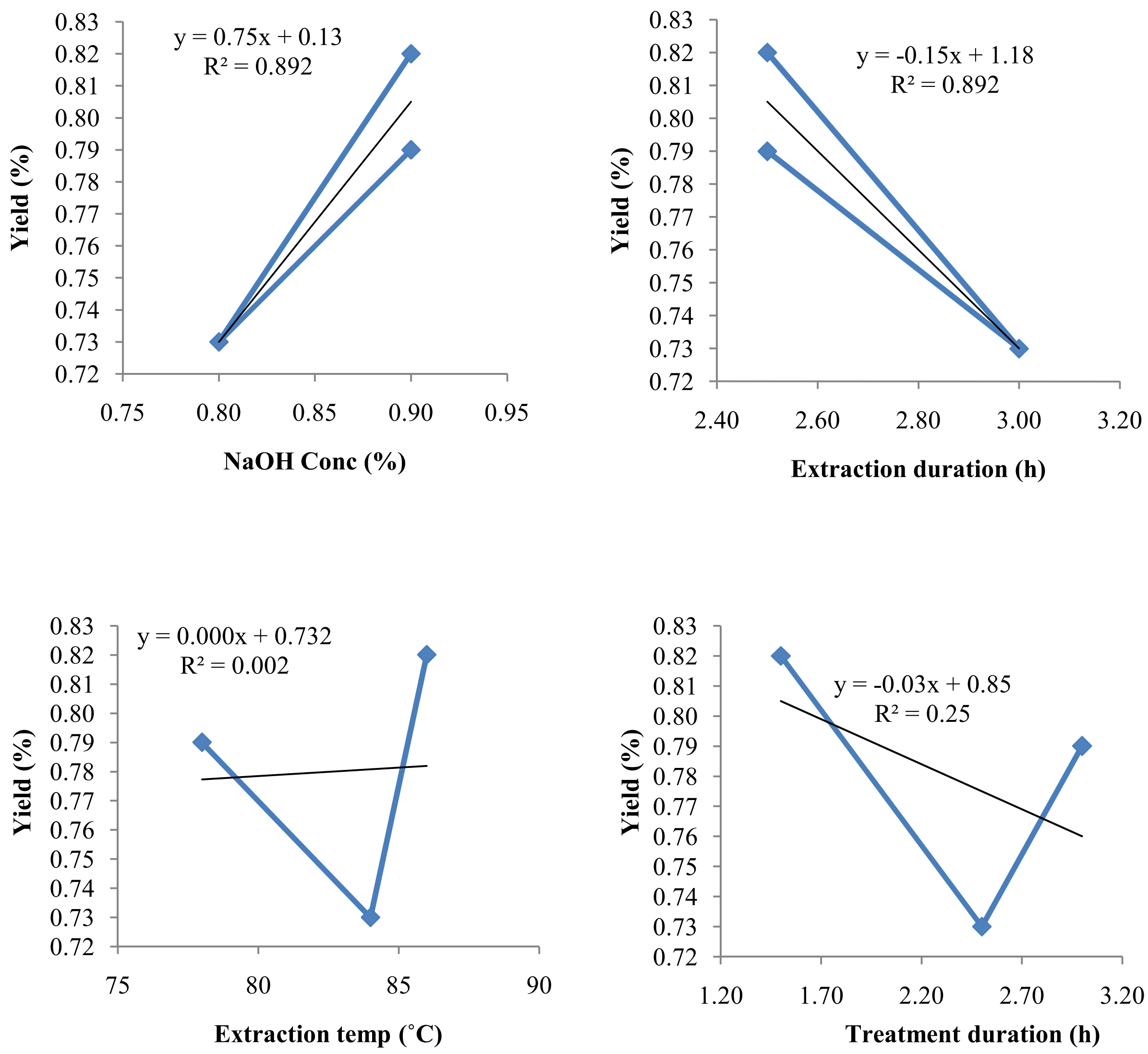


Figure 5: Relationship between yield (%) of gelatin and the concentration of NaOH (%), extraction duration (h), extraction temperature (˚C) and treatment duration (h) from the scales of grass carp.

3.4D Relationship between gelatin Yield (%) with the Concentration (%) of NaOH, Extraction duration (h), Extraction temperature (˚ C) and Treatment duration (h) in Mixed scales.

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

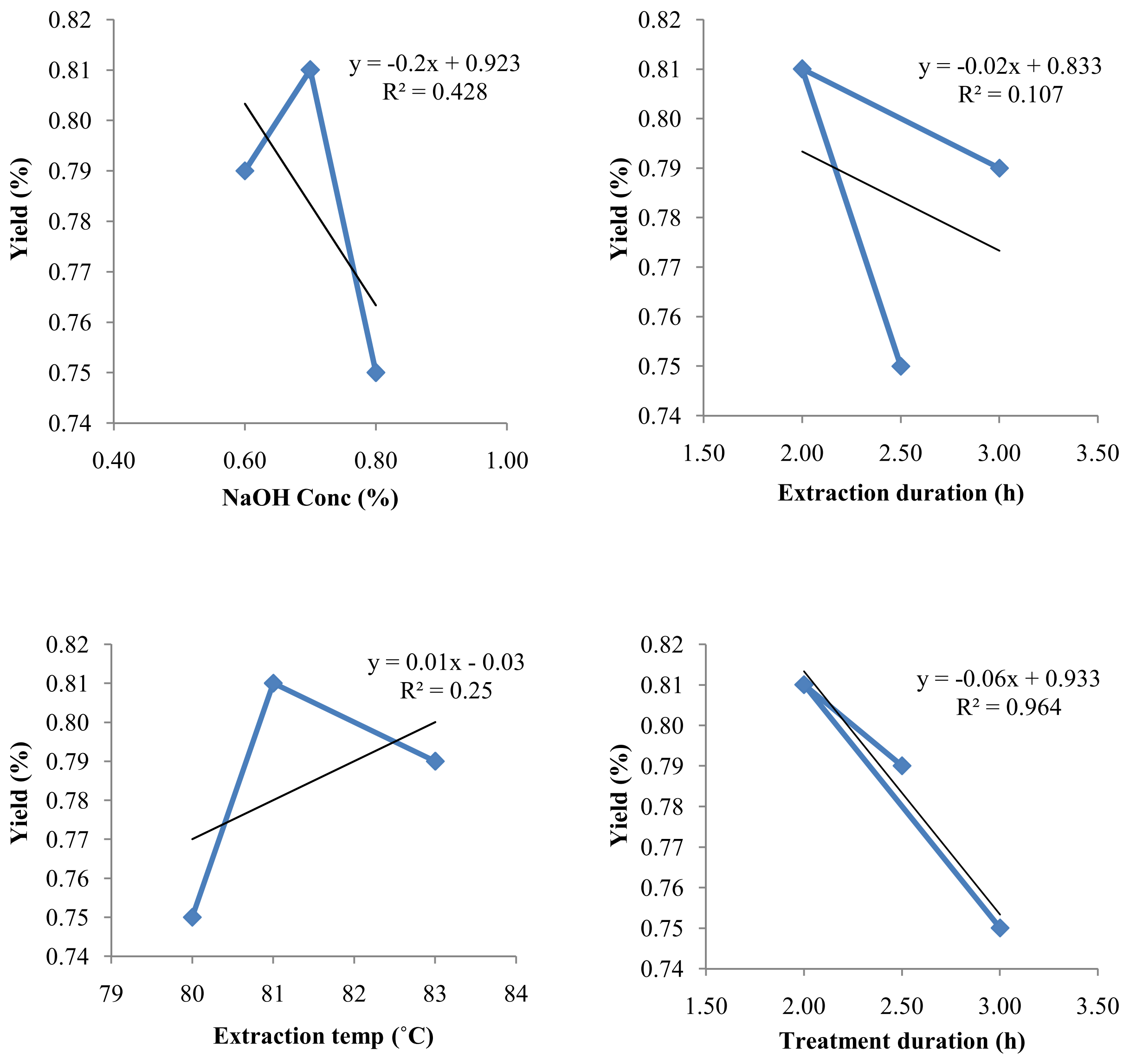


Figure 6: Relationship between yield (%) of gelatin and the concentration of NaOH (%), extraction duration (h), extraction temperature (˚C) and treatment duration (h) from the scales of mixed types of scales.

Chapter 4

Discussion

The yield of gelatins obtained from different freshwater fish species of this study is presented in Figure 2. The highest percentage of gelatin yielded from grass carp with 0.78% of its fish scale. Mixed types of scales had a same level of gelatin yield. Gelatin yielded from catla and rohu were 0.72% and 0.61% respectively. The difference in the gelatin yield could be due to the difference in the characteristics of the skin and 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 lizardfish scale was 10.6% reported by Wangtueai *et al.* (2009). Zeng *et al.* (2010) found 19.3% gelatin yield obtained from tilapia skin. Dinçer *et al*. (2015) found 18.49% gelatin yield obtained from scales of farmed sea bass (*Dicentrarchus labrax*). The low yield in the present study could be due to poor extraction methods.

The amino acid composition of gelatin from catla and rohu fish scales are shown in Table 3. The gelatins developed in this study contained 14 amino acids, with Glutamic acid being the most predominant one. The imino acid (Proline and Hydroxyproline) and Glycine contents of the lizardfish scales gelatin were 20.4 and 18.3%, respectively as reported by Wangtueai *et al.* (2009). The content of imino acid and glycine are important for gel strength. A low content of imino acids indicates poor gelling power (Ward & Courts, 1977). But no imino acid was found in this study neither Proline nor Hydroxyproline. 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. Wang *et al.* (2008) reported lower protein content in silver carp scale (37.91%). Matmaroh *et al.* (2011) reported 43.43% protein in common carp scale and 34.46% protein in golden goatfish. Dinçer *et al*. (2015) reported 96% protein in farmed sea bass scale. The changes in protein percentage values depend on the species.

The effects of four independent variable like concentration of NaOH (%), treatment time (h), extraction temperature (˚C) and extraction time (h) were determined. In catla the yield of gelatin was proportionally related with NaOH concentration (%) and extraction temperature (°C) but inversely related with extraction duration (h) and treatment duration (h). In case of rohu the yield of gelatin was only proportionate with NaOH concentration (%) and inversely related with extraction temperature (°C), extraction duration (h) and treatment duration (h). The yield (%) of gelatin in grass carp had a proportional relation with NaOH concentration (%) and extraction temperature (°C) but inversely related extraction duration (h) and treatment duration (h). 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 (h) and treatment duration (h).

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

Extraction of gelatin from fish scales is important in the context of Bangladesh as tons of fish are cut in the market particularly the big ones namely catla, rohu and grass carp. Tons of fish dry scales are being exported from Bangladesh every year. This huge quantity of dry scales can be used as the raw materials in extracting the fish gelatin. Because fish gelatin is Halal instead of most commercial gelatins are extracted from pig skin and bones. Yield of gelatin from fish scales is another important aspect of extraction. Because if yield is not cost-effective and profitable, no reason to go for that. Therefore, there is a need to optimize the extraction protocol to maximize the yield in quantity not in percent. Low yield in this study could be due to poor extraction methods.

Although yield of gelatin found in this study is low compared to other findings, this can be increased and optimized through improving the extraction methods. Yield of mixed scale had maximum gelatin content which indicates that mass or mixed type of scales drawn from the market can be used profitably as a source of fish gelatin in Bangladesh. However, information presented in this study will provide the basis for further studies.

Amino acid composition of the gelatin found in catla indicates its better 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.

5.2 Recommendations

Only single study was found on the optimization and characterization of gelatin from lizard fish scale. This study perhaps to be the first one in Bangladesh. Therefore, there was a lack of optimum protocol for the extraction of gelatin from fish scale. Therefore, considering the above mentioned points the followings are the recommendations for further studies:

1. Need to quantify the total harvest of dry scales from all fish markets in Bangladesh.
2. Need to develop a standard technique of extraction fish gelatin from fish scales.

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Appendices

Appendix A

Mean yield (%) with 1 SEM variability of gelatin extracted from catla, rohu, grass carp and mixed type scales

				Percent
Fish	Catla	1		.60
		2		.82
		3		.75
		Total	Mean	.7233
			SEM	.06489
	Rohu	1		.69
		2		.52
		3		.63
		Total	Mean	.6133
			SEM	.04978
	Grass Carp	1		.79
		2		.73
		3		.82
		Total	Mean	.7800
			SEM	.02646
	Mixed Scales	1		.75
		2		.81
		3		.79
		Total	Mean	.7833
			SEM	.01764
	Total	Mean		.7250
		SEM		.02792

Appendix B

1-Way ANOVA

Percent

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.057	3	.019	3.273	.080
Within Groups	.046	8	.006		
Total	.103	11			

Appendix C Multiple comparisons

Percent

	Fish	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	Rohu	3	.6133
	Catla	3	.7233
	Grass Carp	3	.7800
	Mixed Scales	3	.7833
	Sig.		.096

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.