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## Effect of pretreatment on physical properties of yellow fin tuna (*Thunnus albacares*) fish glue

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### Abstract

The present study was conducted to evaluate the effect of pretreatment on quality of fish glue made from Yellow fin tuna (*Thunnus albacares*; YFT). Fish glue was prepared by extracting gelatin from fresh and frozen skin of YFT by modified alkaline pretreatment followed by acid pretreatment method. The effect of three different concentrations (0.1, 0.2, and 0.4%) of NaOH and HCl on physical properties was tested at two different soaking time 18 and 24 h using a 3×2 factorial design. YFT skin had a crude protein content of 60.95±0.047%. The best open time (197±1.0 min), desirable time to tack (1.83±0.28 min), high bonding power (24.17±7.48 PSI) at 23 °C soaking temperature and 90 °C extraction temperature, and desirable color (yellowish brown) for YFT fish glue were observed in 0.2% NaOH and HCl treatment with 24 h soaking time. The pH of final glue sample ranged between 3.96 and 5.15. Effect of acid, base concentration and soaking time were significant ( $p < 0.05$ ) on open time and bonding power. The results showed that YFT fish skin can effectively be used for fish glue production using mild acid and alkaline treatments.

**Keywords:** fish glue, collagen, pretreatment, physical property, open time.

### 1. Introduction

Fish glue is a proteneous material extracted from the skin of deep cold water non oily fish such as Cod, Pollock, Haddock. Usually fish glue is a water soluble hydrophilic colloidal protein derived by controlled hydrolyzation of water insoluble collagen in fish skin. Fish glue consisted with several unique features for instance liquid in room temperature, completely water soluble but not in carbonic solvent and it shows excellent adhesion to metal, rubber, glass, leather, wood and paper. Discards from fish processing are generally considered as low valuable resources with negligible market value. It has been reported that 30% of fish waste are in the form of bone and skin with high collagen content. Specially consist with type I collagen according to the Schrieber and Gareis, 2007<sup>[19]</sup> classification.

Fish skin appears to possess a ground plan cross helical fiber of collagen embedded in is the dermis. Collagen is the most abundant protein of animal origin, representing approximately 30 % of total animal protein<sup>[16]</sup>. Collagen is also the major structural protein component of skin, bone, tendon and cartilage. According to the classification of gelatin by Schrieber and Garesis, (2007)<sup>[19]</sup> type I collagen can be widely found in skin, bone, tendon. Type I collagen which contains no Cysteine, is used in the manufacture of gelatin (Cole, 2000). Collagen fibrils are maintained and stabilized by covalent cross links between tropocollagen molecules. The number and type of chemical covalent cross bonds between these chains are altered as the animal age cause the molecules properties of the resultant gelatin

The gelatin production procedure should meet the requirements of removal of non-collagenous materials without alteration to collagen, control the hydrolysis of collagen to gelatin and also recovery and dry of finished product in order to avoid the adverse effect on final yield and finished product. The degree of collagen cross linking is a key factor in decide the pretreatment process required for gelatin manufacture and is highly dependent on a number of factor for such as collagen type, animal species, age. Fish skin contains an important source of highly insoluble collagen which containing low concentration of intra and inter chain non reducible cross links<sup>[12, 13, 14]</sup>. Therefore, mild acid pretreatment is usually used for gelatin production<sup>[18]</sup>. Specially type I collagen extracts from yellow fin tuna fish skin by alkaline pretreatment followed by acid pretreatment and hot water extraction in acidic pH, such treatment leads to a

type A gelatin with an isoelectric point that can vary from 6.5 to 9 [10]. The access of water to collagen fiber accelerate by increasing of HC ions, this absorbed water is held in by electrostatic forces between charged polar groups called electrostatic swelling [8]. The type and concentration of acid used strongly influence on swelling properties and solubilization of collagen.

Therefore the current study is to evaluate the effect of acid and alkaline concentration and soaking time on physical properties of the fish glue and to identify the best pretreatment condition for modified method of yellow fin tuna fish glue preparation. The effect of three different concentrations (0.1, 0.2, and 0.4 %) of NaOH and HCl on physical properties were tested at two different soaking time 18 and 24 h using a 3×2 factorial design.

## 2. Materials and Method

### 2.1 Materials

The fresh and frozen skin of yellow fin tuna were taken from fresh and frozen fish processing line in global sea foods Pvt Ltd, Badalgama, Sri Lanka and analytical grade chemicals were used in the study.

Fresh and frozen skin of yellow fin tuna were processed to remove the scales and flesh using fish knife and thoroughly washed with clean tap water at room temperature. Finally vacuumed packed skins were kept under chill condition until use.

### 2.2 Extraction of gelatin from dorsal skin

The yellow fin tuna skin was washed under running tap water and cut into small parts and chilled at 2-4 °C until used. The cleaned skin was treated with three different concentration (v/w) of alkali solution (0.1- 0.4% NaOH) at 23 °C in soaking bath for 18 h and 24 h respectively and pH of the solution was maintained at 11.5 with two subsequent change of alkaline solution meanwhile remove the non-collagen protein and subcutaneous tissue after they were swollen. After the alkali treatment, the skin was washed and neutralized with same concentration of HCl (0.1- 0.4%) for 18 h and 24 h and washed. For hot-water extraction, double volume (v/w) of distilled water was added and heated at temperature ranging 53–60 °C for 6 h. The extracted solution was filtered through muslin cloth and the filtered solution was heated at 90 °C concentrated to 45-50% solid and preserved it until check the physical properties.

### 2.3 Measurements of Physicochemical Characteristics

#### 2.3.1 Determination of proximate composition of the fish skin and fish glue

Moisture content (oven-drying procedure), crude protein (N ×6.25), lipid (ether extraction) and ash content were estimated by the AOAC official method (Horwitz, 2000). The analyses were repeated for three times.

#### 2.3.2 Determination of open time

Open time is defined as the period of time remains as it liquid or workable. Normally measure in terms of minutes.

Open time is affected by substrate and adhesive type amount and content therefore, homogeneous condition was maintained. Toona (*Cedrela toona*) wood pieces (5 x 5 x 3 cm<sup>3</sup>) were selected as substrate and same amount of fish glue was applied on smooth surface of the wood and clamped each

other and checked for its workability nature by counting the time in minutes. The analyses were repeated for five times.

#### 2.3.3 Determination of set time/ time to tack

Set time is the time it takes to form an acceptable bond when two or more substrate are combined with an adhesives. The glue from each sample were applied on kraft paper pieces (5 x 5 cm<sup>2</sup>) and immediately laminated the other piece of kraft paper on the coated one. Time was recorded in minutes to require separating the two pieces of paper and when one of the kraft paper was torn as set time or time to tack

#### 2.3.4 Determination of bonding power

The bonding power of each glue type that developed from yellow fin tuna skin were determined by double cantilever beam test. The surface of the toona wood pieces were cleaned by using sand paper (no 01) and equal amount of each glue sample was applied by small brush and clamped the two pieces of wood until made the bond. The unit weight /pressure required to separate the tacked wood pieces (PSI or kg/cm<sup>2</sup>) was measured after 30 minutes and 60 minutes interval with five replicates

#### 2.3.5 Determination of color of the fish glue

After preparation of liquid glue it was evaporated in open pan and final glue sample was obtained. Color of each glue sample were measured and observed by putting them on white back ground and compared with each glue sample.

#### 2.3.6 Determination of pH of the glue

pH of the glue sample was determined according to Mohammad. S.R, *et al.* (2008) [15]. Five grams of each glue sample were dissolved in 20 ml of distilled water and mixed thoroughly. Data were taken by pH meter (THERMO, ORION 3 STAR).

## 3. Results and Discussion

### 3.1 Proximate composition of the fish skin and fish glue

The proximate composition of fish skin and glue was found to vary with the type of tissue examined and the fish type. Yellow fin tuna skin has shown moisture content 26.05%, 60.95% crude protein, 10.55% crude fat and 2.4% crude ash on dry matter basis. Also previous study was shown that the dorsal skin of Yellow fin tuna was contained 56.1% moisture, 6.8% crude fat, 1.0% of crude ash and 33.6% of crude protein [2]. Percentage of fat in Yellow fin tuna is very important when preparation of fish glue, since fish glue is usually developed from the non-oily fish skin such as Hake, Pollock and Cod. According to the present study YFT skin has 10.51% fat. This considerable amount of fat present in tuna skin has shown as layer of oil in fish glue at the preparation. That caused for the offensive odor in glue liquor.

### 3.2 Effects of pretreatments during glue preparation

#### 3.2.1 Use of NaOH and HCl for acid, alkaline pretreatment

Gelatin extraction was done under identical condition with the exception of different pre-treatment condition for fish glue preparation described in table 1. Treatment 1 is corresponding to 0.1% HCl acid and 0.1% NaOH concentration for 18 h soaking time. Treatment 2 is corresponding to 0.2% acid and alkaline concentration for 18 h soaking time. Treatment 4, 5, 6 were included same concentration with 24 h soaking time.

**Table 1:** Definition of Treatment

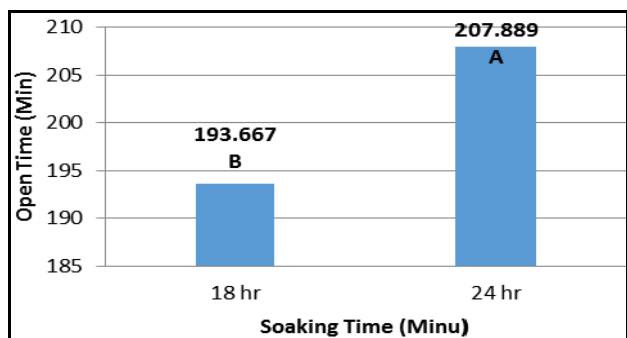
Treatment	Description	
	Acid and Alkaline Concentration (%)	Soaking time (hr)
TR1	0.1	18
TR2	0.2	18
TR3	0.4	18
TR4	0.1	24
TR5	0.2	24
TR6	0.4	24

Treatment is corresponding to combination of HCl, NaOH and soaking time, there were two soaking time in the treatment

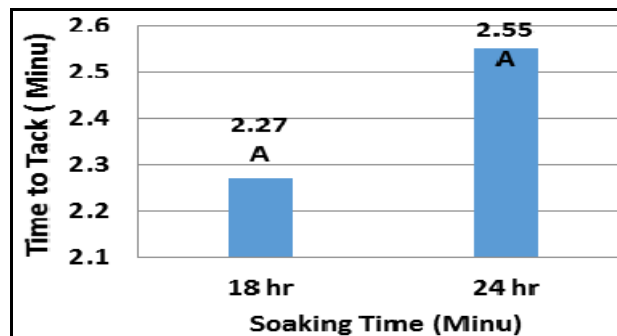
**3.3 Effect of acid and alkaline concentration on physical properties of the fish glue**

The physical properties of the fish gelatin, glue are crucial in its application. Open time, time to tack, bonding power are very important for fish glue because these properties directly related to its application in industry. Physical properties of fish gelatin and glue can be change due to several factor such as raw material, extraction process and amino acid /protein content of the gelatin and glue. Gel strength is one of the important properties of the fish gelatin and the purpose of gelatin was determined by the range of gel strength value. Usually high gel strength value preferable for fish glue specially for bonding power, time to tack. Generally fish gelatin has low gel strength than mammalian gelatin [17]. Especially, characteristics of collagen have influenced on physical properties of gelatin and glue. Go'mez Guille'n *et al.* (2002) reported that tropical fish such as tilapia was a superior material for gelatin processing but yellow fin tuna skin gelatin was shown remarkable gel strength similar to mammalian gelatin [2].

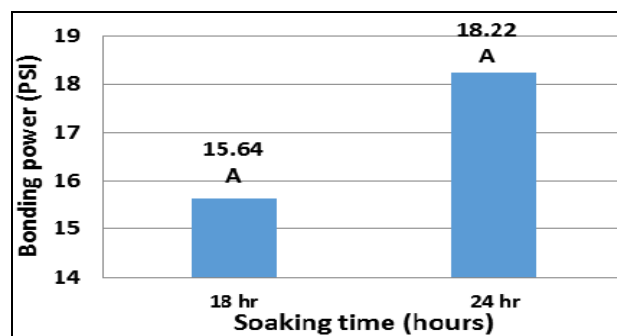
The effect of soaking time on open time, bonding power and time to tack of yellow fin tuna fish glue was determined in this study. It was clearly shown that 18 h and 24 h soaking time for dorsal skin part at 22 °C was not strongly affected on its physical properties. During the soaking time the pH of the soaking solution was maintained at higher level. Increase of soaking time caused to remove the epidermis of the skin effectively and reduce the fishy odor. Open time, against to soaking time was found to be significant indicating differential efficacy of the treatment. Open time in 24 h soaking time was higher (207.8 min) than that of 18h soaking time (193.6 min) (figure 1) whereas commercially available fish glue shows 1.5 to 2.0 h open time [3].



**Fig 1:** Mean open time against soaking time

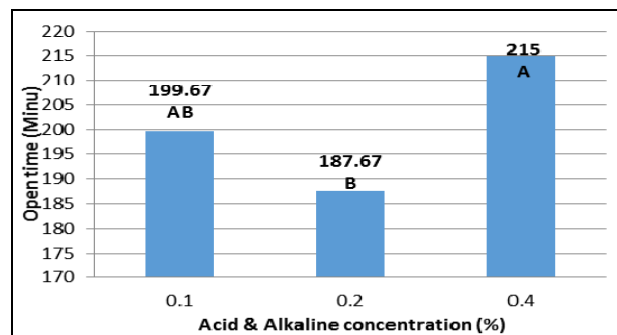


**Fig 2:** Mean Time to tack against soaking time

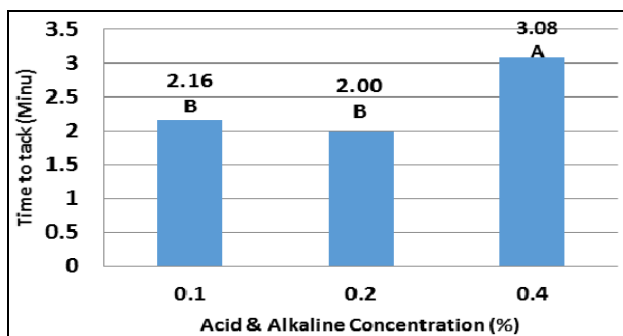


**Fig 3:** Mean bonding power after 30 minutes against soaking time

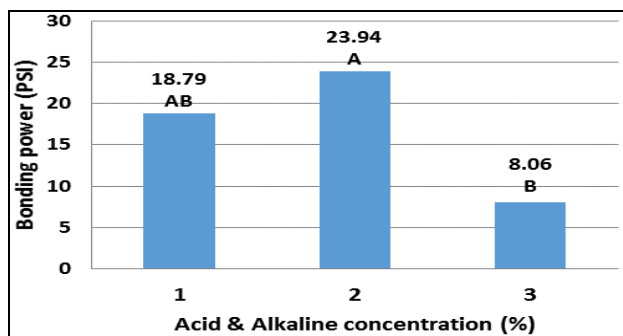
Even though there was significant difference shown in open time, mean values of time to tack and bonding power after 30 minutes were not shown significant difference at ( $p < 0.05$ ). The highest time to tack was shown at 24 h soaking time (2.55 min) and lowest at 18 h soaking time (2.27). Generally lowest time to tack is considered as best, because within very short time it makes good bonds between substances. The commercially available fish glue KREMER shows lesser time to tack compare to yellow fin tuna product (1 min). This may be due to raw material type. Kremer fish glue made out of cold water fish species. Also bonding power was found to be not significant against soaking time. The highest bonding power after 30 minutes shown by fish glue taken by 24 h soaking time (18.2 PSI) followed by 15.64 PSI in 18h soaking time. (Figure 2, Figure 3 ). In this study bonding power was taken at 30 minutes interval. In both treatment it is shown increased mean bonding power 29.7 PSI, 49.3 PSI respectively after 60 minutes. Fish glue has excellent remoistening properties. This allows for easy re-activation of adhesive by water at a later time for bonding [3]. The remoistened surface develops immediate tack and may be bonded to many surfaces including steel, glass and wood.



**Fig 4:** Mean open time against acid and alkaline concentration, Mean open time is given in minutes, at three different concentration levels of HCl and NaOH. Means that do not share the same letter are significantly different at  $\alpha = 0.05$ .



**Fig 5:** Mean time to tack against Acid and Alkaline concentration. Means that do not share the same letter are significantly different at  $\alpha = 0.05$ .



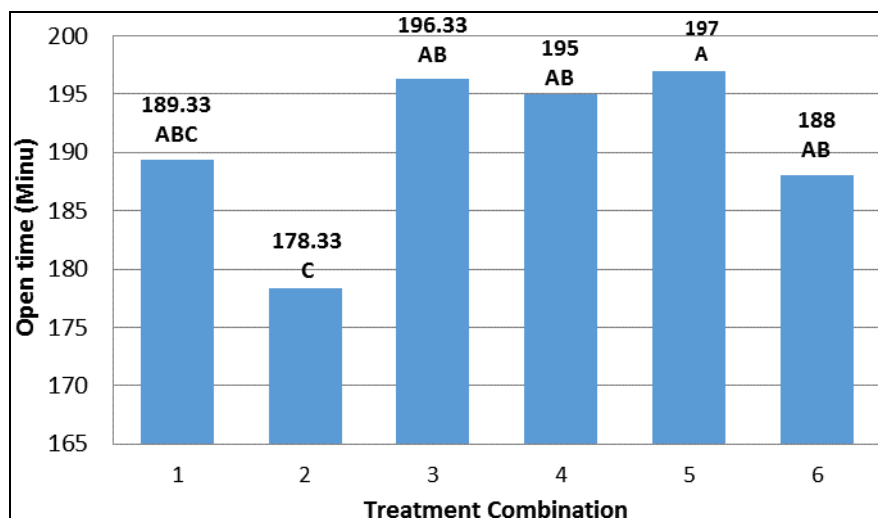
**Fig 6:** Mean bonding power after 30 minutes against Acid and Alkaline concentration. Means that do not share the same letter are significantly different at  $\alpha = 0.05$

According to the results illustrated in figure .4 open time against HCl and NaOH concentrarions were found to be significant ( $P=0.02$ ), indicating differential efficacy of the treatment. The highest open time (215 min) is given at 0.4%

acid and alkaline concentration whereas lowest value (187.67 min) at 0.2% and (199.6 min) at 0.1% concentration level. After 215 minutes, the substrate (Toona wood pieces) could be separated by giving some external force. After separation any adjustment can be made as required and again bond can be made due to its workability and reactivation property. Since it is important to have low time to tack, the lowest mean time to tack value (2.0 min) against acid and alkaline concentration was given by 0.2% while 0.4% level shown highest time to tack value (3.08 min). According to the result, mean time tack value at different acid and alkaline concentration level was significantly difference (Fig.5). Bonding power of fish glue after 30 minutes was given in figure. 6 the highest bonding power (23.9 PSI) was given by 0.2 % acid and alkaline concentration level. It is significantly different from 0.1% and 0.4% Acid and Alkaline concentration level.

The combine effect of soaking time and acid alkaline concentration on physical properties are described in figure 7, 8, 9. According to the results it clearly shows that highest open time (197), highest bonding time (24.17) and second lowest value of time to tack (1.83) was given by treatment 5 corresponds to the 0.2% acid and NaOH concentration for 24 h soaking time.

Even though none of the treatment combinations are statistically significant in bonding power against treatment combinations, it was clearly shown the difference in PSI value at sensory evaluation. Retained time of the bonded substrate also differ. The sample that consisted with high bonding power showed higher retained time (1 year) while other fish glue having low bonding power lasted its bonds after 3-5 months. External force that needed to be separated the bond increased with the time due to re-activation properties of fish glue.



- Tr1 - 0.1 /0.1 +18 h
- Tr2 - 0.2 /0.2 +18 h
- Tr3 - 0.4 /0.4 +18 h
- Tr4 - 0.1 /0.1 +24 h
- Tr5 - 0.2 /0.2 +24 h
- Tr6 - 0.4 /0.4 +24 h

**Fig 7:** Mean open time against treatment combination, Means that do not share the same letter are significantly different at  $\alpha = 0.05$ .

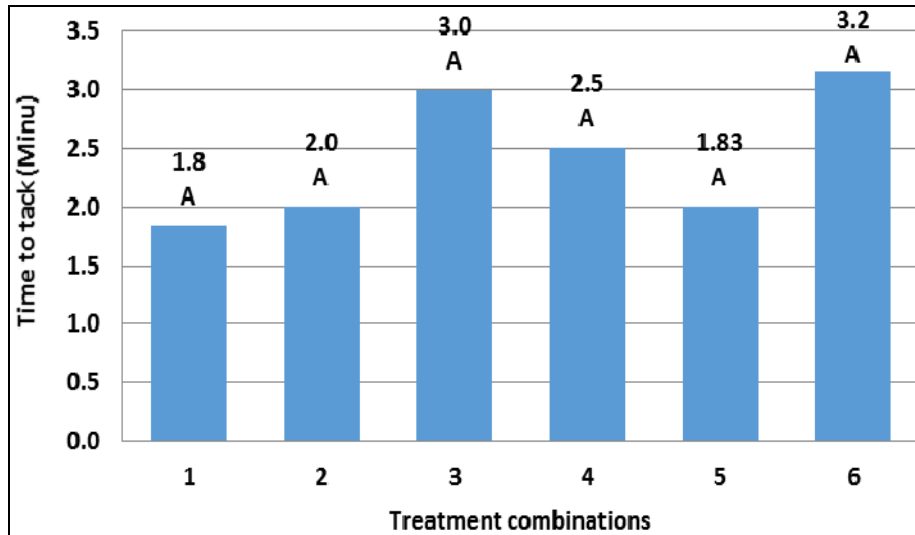


Fig 8: Mean time to tack against treatment combination. Means that do not share the same letter are significantly different at  $\alpha = 0.05$ .

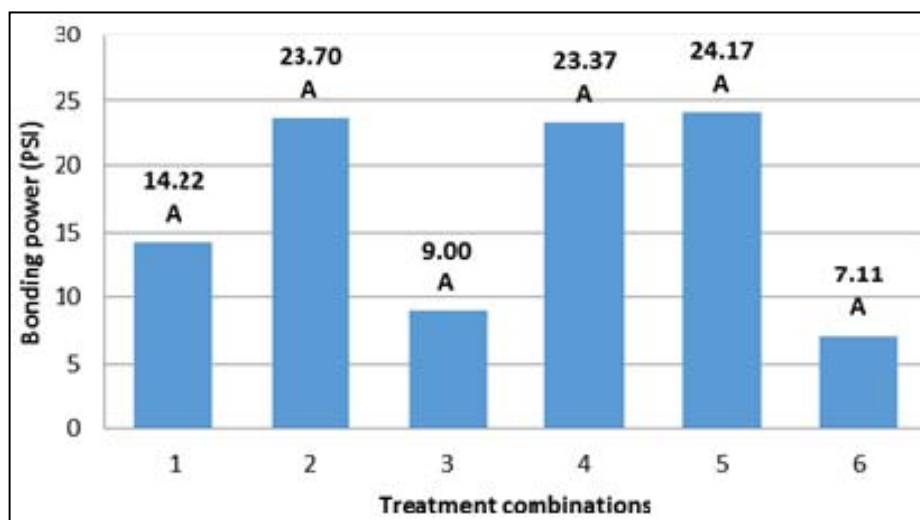


Fig 9: Mean bonding power against treatment combination, Means that do not share the same letter are significantly different at  $\alpha = 0.05$ .

The fish glue preparation is based on the alkaline pretreatment followed by an acid treatments. NaOH or caustic soda, lime ( $\text{Ca}(\text{OH})_2$ ) are the two alkalis that are more often used for gelatin extraction. In this study NaOH was the main alkaline solution. The acid and alkaline concentration of the solution used for pretreatment was shown great influence on the extraction of collagen from fish skin therefore it was not exceeded 0.5 percent sodium hydroxide concentration. Out of three concentration levels 0.4% alkali concentration was shown considerable swelling while resulting minimum swell at 0.1% concentration. Changes in solution was done at 9 h and 12 h interval in soaking time to facilitate the removal of non collagenous material and hydrolyzation process. According to Kernot (1924) <sup>[11]</sup>, the mild alkaline treatment liberates all of the volatile bases which are causative agents of the characteristic fish gelatin odor. This study also revealed that no fishy odor in extracted glue was observed due to the mild alkaline treatment which followed by acid treatment.

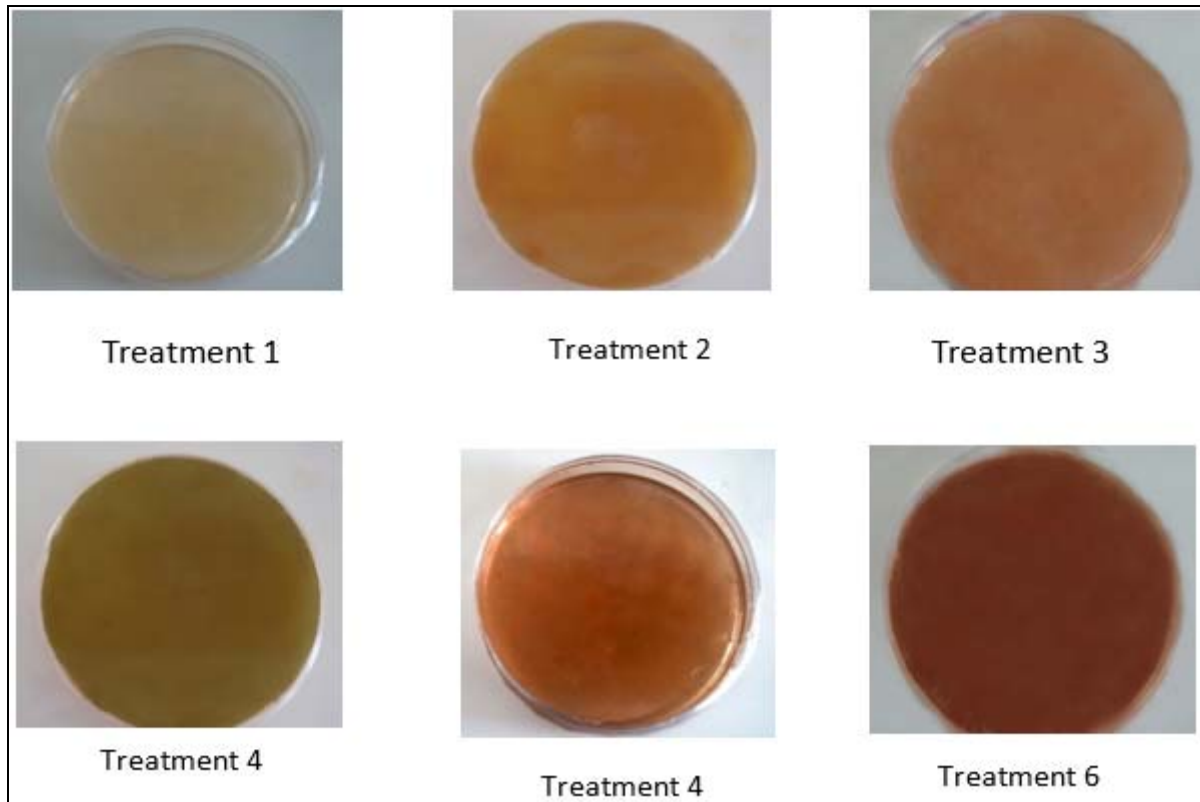
Loss of collagen during 24 hours soaking time period was high compared to the 18 hours soaking time. The lowest swelling ability was shown in order of 0.1% and 0.2% concentration respectively. 0.2% of acid and base concentration was given an effective swelling after 24 hours soaking time which was contributed to retain considerable amount of collagen after

treatments. That implies the importance of low alkaline and acid concentration to avoid excessive swelling of the stock. Previous study of Gudmundsson and Haffsteinson, (1997) <sup>[7]</sup> reported that treating of skin with solution of Sodium Hydroxide and Sulfuric acid concentration higher than 0.2 % and Citric acid above 1% decreased the yield of gelatin and reduced the gel strength. Also Hamid. T. *et al.* (2013) <sup>[9]</sup> reported that effect of NaOH on yield of gelatin has inverse effect, when increase of NaOH concentration it reduces the yield and extracted gelatin but the increase of acid concentration has positive effect on yield and gelatin content. In this study, yield of gelatin was higher in 0.4% and 0.2% HCl acid concentration for 18 h compared to the 0.1 concentration for 24 h soaking time. It also reveals that more washing step reduced the gelatin yield of tuna skin.

### 3.4 Color variation in fish glue

Usually the gelatin extracted from tuna fish skin at different treatment conditions of acid and alkaline pretreatment were given light color solution (figure.10). There were no prominent color variations in gelatin samples. However the tuna glue taken after heat treatment was shown distinct color variation among six treatments.





**Fig 10:** shows the color variance of resulted fish glue from each treatment,

These color variation ranges from amber color to dark red in color. Light amber color was given by the sample of treatment 1 0.1% HCl, NaOH concentration for 18 h soaking time and color was increased with increasing of concentration up to 0.4 at 18 h soaking time. The tuna glue resulted from treatment 4, 0.1% HCl, NaOH concentration for 24 h soaking time was given dark amber color and it changed to dark red in color at high concentration of HCl, NaOH for 24 h soaking time. Reasons for changing the gelatin color is due to pH of the extracted gelatin solution and temperature of the extraction process. It facilitates the millard browning reaction. According to the modified gelatin extraction method, 55-60 °C temperature for extraction and 90 °C, 5-6 hour time temperature combination were used to obtain a glue with 45-55% total solids. Millard browning reaction produces a color pigments called melonimidine which is responsible for the reddish brown to dark brown color of the gelatin. High pH level causes to increase the rate of millard browning reaction vice versa. Final pH levels of the fish glue is given in table 2. According to the results highest pH is shown in treatment 3 and 6 ( $5.15 \pm 0.02$ ) that has dark reddish color fish glue.

**Table 2:** pH value variation in final glue

Treatment	pH of the glue liquor
0.1/18T	$3.96 \pm 0.04$
0.2/18T	$4.31 \pm 0.04$
0.4/18T	$5.15 \pm 0.06$
0.1/24 T	$3.96 \pm 0.04$
0.2/24T	$4.30 \pm 0.01$
0.4/24T	$5.15 \pm 0.02$

pH of the glue sample was determined according to Mohammad. S.R, *et al.* (2008). Determinations were performed in triplicate and data correspond to mean values. Standard deviations were in all cases lower than 0.06.

The light color of the fish gelatin was changed during the evaporation at 90 °C for 6-8 hours. Zhang *et al.* (2007) [20] reported that pretreatment at acidic solution prior to the extraction resulted in transparent gelatin, whereas pretreatment at basic solution resulted in dark colored gelatin from Channel Catfish. Since long reaction time favors the millard reaction between protein and traces of carbohydrates in the raw material, increase of extraction time with high temperature affect gelatin color [19].

#### 4. Conclusion

Yellow fin tuna fish gelatin effectively can be transferred in to the fish glue at high temperature. HCl acid and NaOH concentration showed a clear effect on final fish glue quality especially open time, time to tack and bonding power, therefore mild acid and alkaline treatment is very important. Effect of soaking time on physical properties except open time is low. Fish glue with high bonding power, low time to tack, higher open time and favorable color could be obtained by 0.2 % acid and alkaline concentration for 24 h soaking time. Since fish glue is natural and organic adhesive it is require to add preservative to increase the self -life more than 6 months.

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## APPENDIXES

Figure: Flow chart of fish glue preparation

