

# Extraction of gelatin from catfish bone using NaOH and its utilization as a template on mesoporous silica alumina

R Nuryanto<sup>1,2</sup>, W Trisunaryanti<sup>1</sup>, I I Falah<sup>1</sup>, Triyono<sup>1</sup>

<sup>1</sup>Department of Chemistry, Faculty of Mathematics and Natural Sciences Gadjah Mada University Jl Sekip Utara Yogyakarta, Center of Java Indonesia 55281.

<sup>2</sup>Department of Chemistry, Faculty of Sciences and Mathematics, Diponegoro University, Tembalang Semarang

E-mail: [nuryantorahmad@yahoo.com](mailto:nuryantorahmad@yahoo.com)

**Abstract** Gelatin extraction from catfish bone using NaOH and its utilization as a template on a synthesis of mesoporous silica-alumina had been investigated. The extraction was prepared by immersing 25 g catfish bone in 125 mL of NaOH in concentration of 0.0; 0.05; 0.10; 0.15 and 0.20 M for 24 h, then washing with demineralized water until pH 7, followed by immersed the bone into 125 mL of 1 M HCl for 1 h, then washed using demineralized water into pH 5. To produce gelatin the bone was refluxed with 100 mL demineralized water at 70°C for 5 h then evaporated at 50°C. The dry gelatin was characterized using FTIR and electrophoresis (SDS-PAGE). The best performance of gelatin was produced by NaOH 0.10 M. The gelatin consists of amide A, B, I, II, III and molecular weight of 25-200kDa. Silica and Alumina material prepared from Lapindo mud extraction. Dry Lapindo mud crushed and filtered until pass 100 mesh, then reflux using 6 M HCl (1:4 w/v) at 90°C for 5h then filtered. The filtrate was consisting alumina solution adding with 6 M NaOH (2/3 V/V) them filtered. The filtrate then injected by CO<sub>2</sub> gas for 30 minutes and filtered, the residue was calcined at 500°C for 5h. The residual of Lapindo mud dried and refluxed with 6 M NaOH (1:4 w/v) at 90 °C. After 5h filtered and the filtrate added by HCl to pH 8 and filtered, the residual then dried. The Si and Al were then analyzed by XRF and consist of silica and alumina for 99.1 and 87.73%, respectively. Silica-alumina was prepared using silica and alumina extracted from Lapindo mud. 6 g of SiO<sub>2</sub> and 2 g of NaOH was immersed in 62 mL of demineralized water then added with alumina solution (0.204 g alumina in 30 mL demineralized water). The gelatin solution (5 g gelatin in 70 mL demineralized water) was dropped into the silica-alumina while stirring at 50°C for 4 h and aging for 24 h. The synthesized silica alumina was analysed using FTIR and surface area analyser. The FT-IR spectra indicated the TO<sub>4</sub> (T=Si, Al) vibration at wave number of 1049.28 and 1103.23 cm<sup>-1</sup>. The synthesized silica-alumina showed mesoporous characters with a pore diameter of 41.18 nm and surface area of 32.76 m<sup>2</sup>/g

## 1 Introduction

As the global demand for gelatin is continuously on the rise, many potential sources are being sought for combating this growing need. Majorly derived from pig skin, bovine hides, bones and others sources contributing 46%, 29.4%, 23.1% and 1.5%, respectively [1]. Gelatin is a mixture a mixture of peptides and protein produced by partial hydrolysis of collagen. The quality of gelatin depends on its physicochemical properties, rheological properties, and severity of the manufacturing method [2].



Extraction of fish gelatin from several fish such as cod [3], have skin [4], red and black tilapia [5] red snapper and grouper [2] African catfish [6] has been reported.

Technically, the term gelatin applies for a series of proteins obtained from collagen after partial hydrolysis, obtained from bones, skin, hides, ligaments, and cartilages, etc. In the conversion process of collagen to gelatin, acid or alkali pretreatment hydrolyze the crosslinking bonds between polypeptides and irreversible results in gelatin [7]. The gelatin is water soluble and forms thermo-reversible gels with the melting temperature near to the body temperature [8].

Mesoporous silica–alumina (MSA) belong to the frequently studied systems because their acidity allows various applications as catalysts and carriers. In contrast to zeolites, their characteristic properties are the amorphous character and larger pore diameters. These materials have often been synthesized by the sol–gel methods from organometallic precursors like tetraethyl orthosilicate (Dynasil-A) and aluminum alkoxides in the presence of pore-regulating agents, mostly tetrapropylammonium hydroxide (TPA-OH) and amines [9]. Gelatin from bovine as a template on silica mesoporous has investigated. The porous size of silica mesoporous is 6.08 nm [10].

Gelatin is a potential polymer to be used as a template for synthesis of mesoporous silica because it contains N-H functional groups tend to strongly interact with xylanol groups (Si-OH) on the silicate species via multiple hydrogen bonding. The pH of the solution can affect the concentration of ammonium ions inside gelatins molecules. Moreover, gelatin has good biocompatibility, surf activity biodegradability and non-toxic [10].

## **2 Material and methods**

Catfish bone from home industry on kampung lele, Boyolali, Central Java. Lapindo mud from Sidoarjo, East Java. NaOH, HCl and acetic acid analytical grade were obtained from E-Merck (Germany)

### **2.1 Extraction of Gelatin**

The extraction of gelatin was carried out according to Zelechowska *et al.* [11] and Zou and Regensteins [12] with slight modification. The bones were cleaned and washed with aquadest. Before gelatin extraction, 25 g of catfish bones was immersed with 0; 0.05; 0.10; 0.15 and 0.20 M NaOH solution (1:5, w/v) for 24h. After that, the mixture separated, and the solid portion was washed with aquadest into pH=7; then immersed with 1 M HCl (1:2.5, w/v) for 0.5h were repeated twice. The pretreated catfish bones were then refluxed in demineralized water (1:4, w/v) for 5 h at 70°C The mixture was filtered and filtrate dried at 50°C to produced gelatin. The gelatin was then characterized by FTIR and SDS-PAGE.

### **2.2 Extraction of Si and Al from Lapindo Mud.**

Lapindo mud crushed and sieved with 100 mesh sieve to pass 100 mesh material. Then 75 g material reflux by 6 M HCl (1:4 w/v) at 90 °C for 5 h, then filtered. The Filtrate mixed with 6 M NaOH (2:3 v/v) then filtered. The liquid portion injected with CO<sub>2</sub> for 30 minutes and filtered, the residue was calcined at 500°C for 5h. The residual of Lapindo mud dried and refluxed with 6 M NaOH (1:4 w/v) at 90 °C. After 5h filtered and the filtrate added by HCl to pH 8 and filtered, the residual then dried. The Si and Al were then analyzed by XRF.

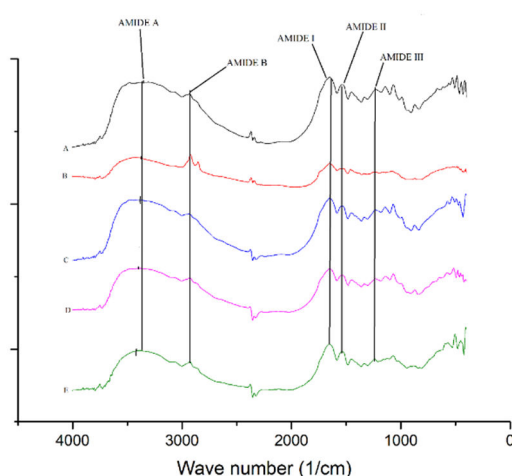
### **2.3 Synthesis of mesoporous Silica-alumina.**

The synthesis began by mixed 6 g SiO<sub>2</sub> and 2 g NaOH dissolved in 62 mL demineralized water and stirred. 0.204 g Al<sub>2</sub>O<sub>3</sub> dissolved in 30 mL demineralized water. Alumina solution was added to the silica solution. The gelatin solution was made from 5 g gelatin (immersed by NaOH 0.1 M) dissolved in 70 mL demineralized water at 50°C then added in the silica-alumina solution drops by drops while stirred for 30 minutes and aged for 24 h. After that mixture of Silica Alumina added with HCl drops by drops into pH 10.5 while stirred for 30 minutes, then filtered and solid dried in the oven 120°C for 24 h. Finally, the material calcined in the furnace at 550°C for 5 h, then analyzed by FTIR, GSA and XRD.

### 3 Result and Discussion

#### 3.1 Characterization of Catfish bones Gelatin

Figure 1. All gelatins was showed five characteristic FTIR polypeptide band namely amide A, B, and I-III. Amide I band  $1651\text{cm}^{-1}$  is mainly due to C=O stretching vibration coupled to contribution from the CN stretch, CCN deformation and in-plane bending modes [13]. The amide II band exhibited at a wave number of  $1527\text{cm}^{-1}$  is a resulted from an out of phase combination of a CN stretch and in-plane NH deformation modes of peptide group [13] [14]. The amide III were observed at wave number  $1234\text{cm}^{-1}$ , indicated the disorder in the gelatin molecules and were more likely associated with the loss of triple helix state [15][16]. The amide III bands represent the combination peaks between CN stretching vibrations and NH deformation from the amide linkages as well as the absorptions arising from wagging vibrations of  $\text{CH}_2$  groups in the glycine backbone and proline side-chains [17].



**Figure 1.** FTIR Spectra of catfish bone Gelatin with immersed in NaOH (A) 0.0 M (B) 0.05 M (C) 0.10 M (D) 0.15 M (E) 0.20 M.

The amide A band was found at  $3387\text{cm}^{-1}$  assisted with the stretching vibrations of NH group coupled with hydrogen bonding. Normally, a free NH stretching vibrations are found in the range of  $3400\text{--}3440\text{cm}^{-1}$ . The position of this band shifted to more allow frequency because the NH group of a peptide is involved in hydrogen bonding [18]. Amide B was observed at  $2931\text{cm}^{-1}$  corresponding to the asymmetric stretching vibrations of C=H as well as  $\text{NH}_3$  [16].

**Table 1:** Molecular weight distribution of gelatin

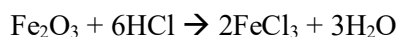
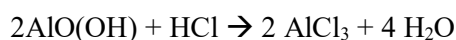
NaOH concentration (M)	Molecular weight range (kDa)
0.00	Not recorded
0.05	15-200
0.10	25-200
0.15	< 10
0.20	<10

Table 1 showed the molecular weight distribution of extracted gelatin. The weight distribution was calculated with standard curve of SDS-PAGE data. Extracted gelatin with immersed in NaOH 0,05 M and 0,10 has molecular range 15-200 kDa and 25-200 kDa, respectively. The other NaOH concentration has a molecular weight less than 10 kDa. Gelatin extracted with immersed NaOH 0,1 M chosen with template because It has molecular weight higher than gelatin from immersed NaOH 0.05 M. The material is termed gelatin when having a minimum molecular weight of 30 kDa [10].

### 3.2 Extraction of Si and Al from Lapindo mud.

Extraction of Lapindo mud to obtain Si and Al, because it contains 46.7 % Si and 13 % Al [19]. The main purpose of acid extraction is to destroy the crystalline aluminosilicate phase, such as kaolinite, and boehmite, to release free aluminum ions. According to the thermodynamic calculation at room temperature [20], but the reaction rate is so slow that the aluminum minerals can be thought of as inert to acids at room temperature. Thus, the high-temperature acid leaching method was adopted, and hydrochloric acid was chosen as the leaching agent.

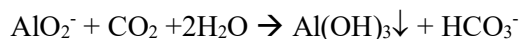
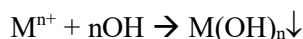
Another metal such Fe contains in the solution. Metal impurities could remove using precipitation method with added 6 M NaOH. Some impurities will be precipitated into  $M(OH)_n$  except Al, the reaction of precipitation are as follows:



**Table 2.** Yield and purity of Silica and Alumina from XRF analysis

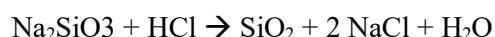
	Silica (%)	Alumina (%)
Yield	24	7
Purity	99.1	87.73

Alumina can precipitate by injected using  $CO_2$  gas with reaction as:



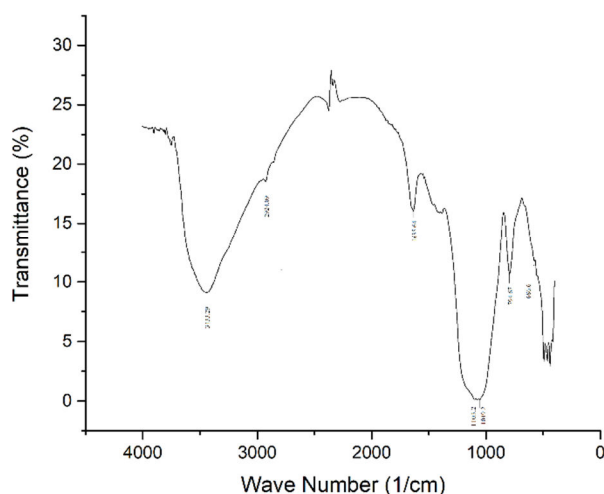
The yield of alumina the extraction is 7 %, and the result of XRF has a purity of 87.73%. Another material included yield are Si 5.94%, Fe 2.35 %, and another material.

Silica was extracted from solid residue alumina extraction, by reflux using NaOH. Solid silica could obtain with added HCl in the filtrate. The yield of Silica from extraction is 24 % with purity of 99.1%, then other material impurities are Fe 0.51% and the other materials. The reactions of extraction are as follow:

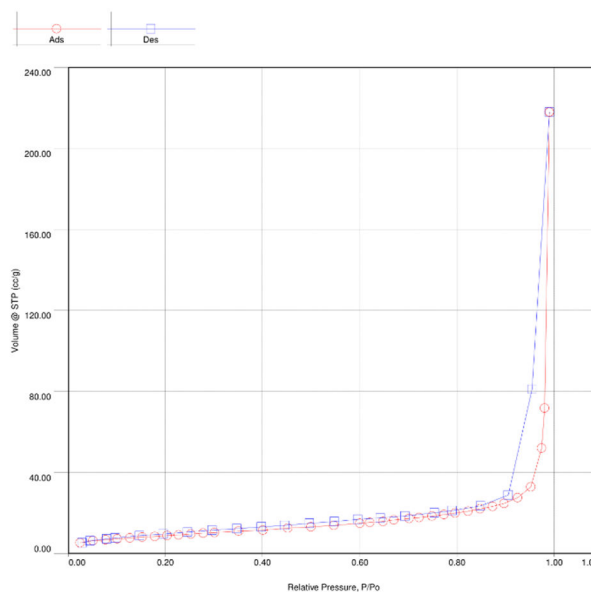


### 3.3 Synthesis of mesoporous Silica-alumina

**Figure 2.** illustrates the FTIR spectra of silica alumina. FT-IR spectra are collected in  $3433\text{ cm}^{-1}$  is correspondent to overlapping of the O-H stretching bands of hydrogen-bonded water molecules ( $H-O-H \cdots H$ ) with  $SiO-H$  stretching of surface xylanols hydrogen-bonded to molecular water ( $SiO-H \cdots H_2O$ ). A band at  $2924\text{ cm}^{-1}$  is symmetric and asymmetric fundamental stretching vibrations of  $CH_2$  and  $CH_3$  groups belonging to gelatin as a template. The band at  $1635\text{ cm}^{-1}$  belongs to the scissor vibration arising from the proton vibration in the water molecule. The bands at  $1049$  and  $686.65\text{ cm}^{-1}$  represent the asymmetric and symmetric stretching vibrations corresponding to the inner  $TO_4$  structure ( $T = Si, Al$ ), respectively, whereas the bands at  $1103$  and  $794\text{ cm}^{-1}$  represent the asymmetric and symmetric stretching vibrations corresponding to the external  $TO_4$  structure ( $T = Si, Al$ ), respectively [21].

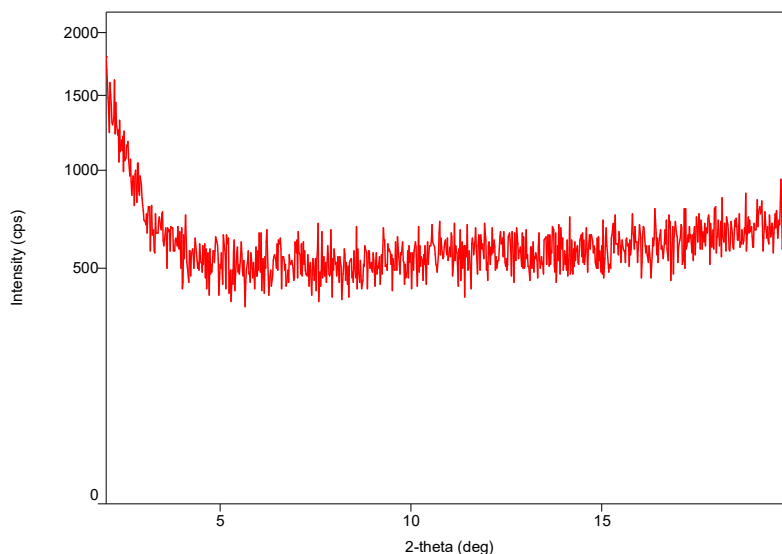


**Figure 2.** FTIR Spectra of Mesoporous Silica-Alumina



**Figure 3.** Nitrogen adsorption-desorption isotherm of mesoporous silica-alumina

Mesoporous indicated by exhibit type IV isotherm with hysteresis loops, indicating textural mesoporous (figure 3). The isotherm of Silica-alumina mesoporous exhibit increases at relative pressure  $p/p_0$  0.9-1.0. The synthesized of mesoporous silica-alumina pore calculated by the BJH Method has pore diameter 41.18 nm. Specific surface area of the silica alumina material was calculated by BET method, the result of a specific surface area was  $32.76 \text{ m}^2/\text{g}$ . Pore volume determines by nitrogen adsorption at relative pressure ( $p/p_0$ ) 0.990, of mesoporous silica alumina was  $0.337 \text{ cm}^3 \text{ g}^{-1}$ . The crystallinity of silica alumina investigated with XRD was presented in figure 4. XRD pattern without peak around line  $2\theta$  so that the silica-alumina was indicating an amorphous material.



**Figure 4.** XRD pattern of Mesoporous Silica Alumina

#### 4 Conclusion

Gelatin extracted from catfish bone by immersion in 0.1 used as mesoporous silica alumina template. An amorphous Mesoporous silica alumina mesoporous has been successfully preparing using catfish gelatin as a template. Based on adsorption-desorption, the Mesoporous Silica Alumina have pore diameter, specific surface area and pore volume of 41.18 nm, 32.76 m<sup>2</sup>/g, and 0.337 cm<sup>3</sup> g<sup>-1</sup>, respectively.

#### Acknowledgement

This research was conducted under the research grant of PUPT 2017 Universitas Gadjah Mada. The authors therefore thank to the Indonesian Ministry of Research, Technology, and Higher Education for the financial support (Contract number: 2454/UN1.P.III/DIT-LIT/LT/2017).

#### References

- [1] Karim, A. A. and Bhat, R. (2009) 'Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins', *Food Hydrocolloids*. Elsevier Ltd, 23(3), pp. 563–576. doi: 10.1016/j.foodhyd.2008.07.002.
- [2] Jeya Shakila, R. *et al.* (2012) 'Functional characterization of gelatin extracted from bones of red snapper and grouper in comparison with mammalian gelatin', *LWT - Food Science and Technology*. Elsevier, 48(1), pp. 30–36. doi: 10.1016/j.lwt.2012.03.007.
- [3] Gudmundsson, M., & Hafsteinsson, H. (1997). Gelatin from cod skins as affected by chemical treatment. *Journal of Food Science*, 62(1), 37-47.
- [4] Gomez-Guillen, M. C., Turnay, J., Fernandez-Diaz, M. D., Ulmo, N., Lizarbe, M. A., & Montero, P. (2002). Structural and physical properties of gelatin extracted from different marine species: a comparative study. *Food Hydrocolloids*, 16, 25-34
- [5] Jamilah, B., & Harvinder, K. G. (2002). Properties of gelatins from skins of fish black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). *Food Chemistry*, 77,81-84.
- [6] Alfaro, A. T. *et al.* (2014) 'African catfish (*Clarias gariepinus*) skin gelatin: Extraction optimization and physical-chemical properties', *Food Research International*. Elsevier Ltd, 65(PC), pp. 416–422. doi: 10.1016/j.foodres.2014.05.070
- [7] Yang, H., Wang, Y., Zhou, P., & Regenstein, J. M. (2008). Effects of alkaline and acid

- pretreatment on the physical properties and nanostructures of the gelatin from channel catfish skins. *Food Hydrocolloids*, 22(8), 1541–1550.
- [8] Norziah, M. H. *et al.* (2009) ‘Characterization of fish gelatin from surimi processing wastes: Thermal analysis and effect of transglutaminase on gel properties’, *Food Hydrocolloids*. Elsevier Ltd, 23(6), pp. 1610–1616. doi: 10.1016/j.foodhyd.2008.12.004.
- [9] Vít, Z. and Šolcová, O. (2006) ‘Synthesis and properties of mesoporous silica-alumina with narrow pore size distribution obtained without use of pore-regulating agents’, *Microporous and Mesoporous Materials*, 96(1–3), pp. 197–204. doi: 10.1016/j.micromeso.2006.06.020.
- [10] Trisunaryanti, W. *et al.* (2016) ‘Extraction of gelatin from bovine bone and its use as template in synthesis of mesoporous silica’, *Asian Journal of Chemistry*, 28(5), pp. 996–1000. doi: 10.14233/ajchem.2016.19561.
- [11] E. Zelechowska, M. Sadowska and M. Turk, Isolation and some properties of collagen from the backbone of Baltic cod (*Gadus morhua*), *Food Hydrocoll.*, 24, 325 (2010)
- [12] P. Zhou and J.M. Regenstein, *J. Food Sci.*, 70, C392 (2005)
- [13] Bandekar, J. (1992). Amide modes and protein conformation. *Biochimica et Biophysica Acta (BBA) – Protein Structure and Molecular Enzymology*, 1120, 123–143.
- [14] Lavalie, F., Adams, R. G., & Levin, I. W. (1982). Infrared spectroscopic study of the secondary structure of melittin in water, 2-chloroethanol, and phospholipid bilayer dispersions. *Biochemistry*, 21, 2305–2312.
- [15] Friess, W., & Lee, G. (1996). Basic thermoanalytical studies of insoluble collagen matrices. *Biomaterials*, 17, 2289–2294.
- [16] Sinthusamran, S., Benjakul, S. and Kishimura, H. (2014) ‘Characteristics and gel properties of gelatin from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions’, *Food Chemistry*, 152, pp. 276–284. doi: 10.1016/j.foodchem.2013.11.109.
- [17] Jackson, M., Choo, L. P., Watson, P. H., Halliday, W. C., & Mantsch, H. H. (1995). Beware of connective tissue proteins: Assignment and implications of collagen absorptions in infrared spectra of human tissues. *Biochimica et Biophysica Acta Molecular Basis of Disease*, 1270, 1–6.
- [18] Doyle, B. B., Blout, E. R., & Bendit, E. G. (1975). Infrared spectroscopy of collagen and collagen like polypeptides. *Biopolymers*, 14(5), 937-957.
- [19] Fadli., A.F., Tjahjanto., R.T., and Darjito, 2013, Ekstraksi silika dalam lumpur lapindo menggunakan metode kontinyu, *Kimia. Student Journal*, Vol. 1, No. 2, pp. 182-187 Universitas Brawijaya Malang
- [20] Dai, H., 1997. Thermodynamics discussion of alumina production by acid method. *Nonferrous Min.Metall.* 4, 40–44 .
- [21] Zhao, J. *et al.* (2016) ‘Synthesis and characterization of mesoporous zeolite Y by using block copolymers as templates’, *Chemical Engineering Journal*. Elsevier B.V., 284, pp. 405–411. doi: 10.1016/j.cej.2015.08.143.