

Characterization of Gelatines Extracted From Cow Bone for Carbon Synthesis

Maria Ulfa¹, Wega Trisunaryanti¹, Iip Izul Falah¹ and Indriana Kartini¹

¹Department of Chemistry, Faculty of mathematics and natural sciences
Gadjah Mada University Jl. Sekip Utara Yogyakarta, Center of Java, Indonesia 55281.

Abstract: Extraction of gelatin from cow bone was carried out using the combination of alkali and acid pretreatment by a sequential extraction process. Characterization gelatin was analysis using infrared spectroscopy (IR), SEM-EDAX, DTA-DSC, and electrophoresis. The best character of gelatin was use for synthesis of porous carbon. Gelatin was extracted in different time, acid concentration and extraction temperature. The IR spectra indicated that gelatin I extracted at shorter time, lower acid concentration and temperature showed amide I and small intensity of amide III bands. Gelatin II (extracted at longer time, higher acid concentration and temperature) showed higher intensity of amide I and III band compare to those of gelatin I. The SEM of gelatin II was also in form that morphology of gelatin II was better than that of gelatin I. The electrophoresis analysis showed that the range of molecular weight of gelatin II was 90-110 kDa containing carbon and nitrogen of 48 and 15 wt%. The porous carbon synthesized by carbonization of gelatin II showed surface area, pore diameter and volume of 89 m²/g, 15 nm and 0.01 cm³/g, respectively

Keywords: cow bone, gelatin, extraction, porous carbon

I. Introduction

Carbon has been extensively used for many applications in industrial and environmental field. The application capability and activity were controlled by their surface characteristics such as surface area, pore diameter and volume. The carbon can be synthesized using various sources such as sugar cane bagasse and sunflower seed hull [1], Camellia oleifera shell [2], sucrose [3], furfuryl alcohol (FA), 4,4'-bismaleimidediphenyl methane (BM), copolymer with divinylbenzene (BM-DVB) [4] and natural polimers like gelatin [5]. The advantages of using gelatin as carbon source are its abundant, high carbon content, low price, biodegradable, and nontoxic. Therefore, the gelatin could be regarded as an alternative source to synthesize the porous carbon. However, the large of molecular weight of the gelatin could be one factor that control the carbon surface characters.

Gelatin was obtained by controlled hydrolysis of collagen molecules [6-7]. The Fourier transform infrared (FTIR) inform that gelatin showed three specific region of amide A (3300-3500 cm⁻¹), amide I (1636-1661 cm⁻¹), amide II (1549-1558 cm⁻¹) and the amide III (1200-1300 cm⁻¹). The heat treatment, acid concentration and duration of extraction time broke hydrogen bonds of triple helix (tropocollagen) from collagens to random coil of gelatin. The hydrolysis process changed the molecular weight and structure of gelatin. This hydrolysis condition also affected the length of amino acid chain and thermal stability of gelatin [8].

Gelatin from various species such as fish, bovine and animal skins from various places have been investigated [9-10]. Gelatin of source of carbon was investigated previously for synthesis mesoporous carbon [11-13]. In the previous the authors have undertaken the synthesis of mesoporous carbon using gelatin extracted from cow bone as carbon source. However, the previous studies have not discuss about utilization of gelatins not only as carbon source but also as soft template the in synthesis of carbon. Based on the above explanation the author investigated the study of characterization of gelatin extracted of cow bone as carbon source as well as soft template for synthesis porous carbon. Gelatin was analysis by infrared spectroscopy (IR), SEM-EDAX, DTA-DSC, and electrophoresis.

II. Experimental Section

2.1 Material

Cow bones were collected from the meat market of Ngawi city (Indonesia). Bones were cut manually into small pieces (1 to 2 cm length) using knife, drying 110 °C for 48 h then kept in the sample box before use. All chemicals used were analytical grade.

2.2. Instrumentation

FTIR spectra for functional group analysis were obtained from discs containing 1 mg sample of gelatin in approximately 30 mg kalium bromide (KBr). All spectra were obtained using a Bruker infrared spectrophotometer (Bruker Instruments, Billerica, MA) from 4000 to 500 cm^{-1} at data acquisition rate of 2 cm^{-1} per point. Triplicate samples of gelatins were analysed and spectra for the triplicate runs averaged. Thermal stability of materials are characterized by DSC-DTA (Q100 TA Instruments). The morphology of the gelatin was examined by means of a scanning electron microscope, Philips, model XL-30. The microscope was equipped with an energy dispersive Si(Li) detector EDAX DX4 for microanalysis. Molecular weight of gelatin analyzed by electrophoretic using amine-protein vertical slab electrophoresis system (Bio-Rad Laboratories, USA) with the stacking and resolving gels contained 5% of acrylamide. The adsorption/desorption of Nitrogen conducted to porous carbon of gelatin at 200 °C for carbon samples. The obtain isotherms use to calculate the specific surface area, pore diameter and pore volume of material.

2.3 Extraction of gelatin from the cow bone

The samples were placed on ice and transported, immediately, to the laboratory. The outside of cow bone was thoroughly cleaned with fresh cold running water. The shell was cut manually. The inside was rinsed with fresh cold running water to remove sand or other impurities. Before gelatin extraction, bone was soaked in 1M NaOH with a bone/solution ratio of 1:5 (w/v). The mixture was stirred continuously for 2 h at room temperature at a speed of 150 rpm using an stirrer magnetic. The alkaline solution was changed every 1 h to remove non collagenous proteins and pigments. Alkaline-treated bone was then washed with tap water until neutral or faintly basic pH of wash water was obtained. The bone was then soaked in 3% chloride acid with a bone /solution ratio of 1:5 (w/v) for 24 h with gentle stirring at 50°C. The acidic solution was changed every 2 h to swell the collagenous material in the fish bone matrix. Acid-pretreated bone was washed thoroughly with tap water until wash water became neutral or faintly basic. To extract gelatin, the swollen bone was soaked in distilled water (55 °C) with a bone/water ratio of 1:7 (w/v) in a temperature-controlled water bath for 4 and 8 h with a continuous stirring at a speed of 200 rpm. The mixture was then filtered using a Whatman No. 42 filter paper. The resultant filtrate was freeze-dried then evaporate at 80 °C for 24 h. the dry gelatin kept on desicator until use to analyze process. The different sample carried out at acid concentration, time and temperature extraction. Gelatin I and II represent the sample extracted at mild and higher extraction condition. A set of gelatin samples prepared at 45-75 °C were labeled as follows: GA, GB and GC, respectively. Sampel Gcom represented commercial gelatin. A set of gelatin samples prepared 75 °C for 2, 7, 12 and 23 h were labeled as follows: GC1, GC2, GC3 and GC4 respectively. The lower molecular weight of gelatin analyzed by electrophoretic then use by source of porous carbon. The produced carbons in this work are identified as CFG.

Carbonization Porous carbon from gelatin

The preparation of porous carbon from gelatin was started with synthesized carbon from gelatin (CFG) using water in-oil emulsion method. The CFG were then dehydrated at 110 °C and 200°C in air for 2 h successively, and exposed to air at 250 °C for 8 h then were dispersed in 100 ml of 0.25 mol/L boiling sulfuric acid solution at 98 °C for 12 h. After filtrating, washing and drying, CFG carbonized in nitrogen at 900 °C for 3 h.

III. Result And Discussion

Gelatin sample of GA and GB (Fig 1.) extracted at 45 °C and 65 °C, exhibited high lower intensity amide I and II bands than gelatin GC which is extracted at 75 °C. The spectra of gelatin GA and GB similar with commercial gelatin (Gcom) which is not only showed low intensity of amida I and II but also the amide III band was almost non-existent The profile of low intensity appear in GC1 and GC2 gelatin sample for short time extraction (2h and 7 h) (Fig 2.). All the sample of mild condition showed higher percent peak area around 1690 cm^{-1} indicating that the triple helical structure of gelatins was affected by extraction process. These changes are indicative of greater disorder in gelatin and are associated with loss of triple helix state. This is consistent with changes expected as a result of denaturation of collagen to gelatin.

The gelatin extracted at the higher temperatures (Gelatin GC) (Fig 1) however, exhibited distinct amide III peaks. It seems therefore, that the extent of order in the high temperature-extracted gelatins may be higher than that in low temperature-extracted gelatins. The gelatins extracted at higher temperature exhibited a much broader amide A than was observed for the low temperature-extracted gelatins. The amide A band in the high temperature extracted gelatins was in fact merged with the CH_2 stretching band expected to occur at around 2930 cm^{-1} . Amide A tends to merge with the CH_2 stretch peak when carboxylic acid groups exist in stable dimeric (intermolecular) associations [14-15]. It seems, therefore, that there are more associated components in the high temperature-extracted gelatins. The high temperature extracted gelatins consist mainly of low molecular weight peptides [16-17]. Gelling of low molecular weight gelatin fractions entails more protein-

protein linkages than gelling of high molecular weight gelatins. During drying, therefore, it seems that the low molecular weight, high temperature extracted gelatin fractions denatured slowly, forming a network with more protein-protein linkages than the high molecular weight low temperature extracts. The differences between gelatin I and II suggested that the chain morphology of gelatin as transition process to get the loss of molecular order as showed at scanning electron microscopy (Fig 7).

Fig 3 showed DTA analysis show a weight loss, associated to the degradation of the organic part, of 35 % and a thermal stability over 500 °C. DTA trace demonstrates that gelatin have weight loss 100% at 1200 °C refers to the completely gelatin decomposition to be carbon, hydrogen, oxygen and nitrogen. As-obtained gelatin were characterized by differential scanning calorimetric (DSC profile Fig 4) to verify the effective formation of structure of the absence of external polymer. In the case of gelatin, external polymer is visible by the presence of glass transition phenomenon, when confined polymer does not show that because polymer chains do not have enough space for collective motions and to change their state. The SEM and EDAX profile Fig 4 and Fig 5 show that the Gelatin prepared from high temperature condition contain about 48% of carbon and 15 % nitrogen (Table 1). For this samples, the N and Si contents were high, owing to the fact that amine group interact with acid by hydrogen bonding during extraction process. Table 1 showed that gelatin sample contents consisted of C, N, O and other elements indicated a relatively small amount of functional groups present on the surface and showed some interaction between gelatin and acid using amine group and anion species via hydrogen bonding interaction. This interaction held on extraction process and disappears on evaporation process. The result of that behavior has been reported previously [18-19] which indicated that several ordered of gelatin structures can be produced by the combination of alkali and acid pretreatment

The electrophoresis profile of the gelatin are shown in Fig. 6. showed the three chains forming the triple helix, except that one obtained in more drastic conditions (>110 , > 24 h °C). Two bands α (α_1 and α_2) were between 115 and 82 kDa and the β band was around 180 kDa. The presence of molecules of high molecular weight (>180 kDa), characteristic of γ components was also observed in most of the gelatin. Bands of lower molecular weight with a weaker intensity were also observed, and aggregates of high molecular weight entrapped in the stacking gel were probably gelatin dimers covalently linked for all treatments, bands α_1 and α_2 were less clear at higher reaction times, while bands of lower molecular weight became more pronounced. Gelatin treated at 45-65 °C showed more clearly the presence of the three bands, indicating a low level of gelatin denaturation as confirmed by DSC results. In addition, a lower amount of low molecular weight molecules was observed, as the treatment was performed at temperature below the collagen renaturation. Gelatin obtained at 75 °C for 23 h respectively did not show any clear band, but a smear between 180 and 37 kDa (Fig 6). Similar results were found for type A gelatin, which is produced by an acid treatment. Gelatin also shows a high amount of lower molecular weight polypeptides which is characteristic of a more drastic hydrolysis process. Gelatin GC (Fig 6) have highest ordered structural of gelatin than the other sample. Gelatin GC used as source of porous carbon in order to get the large diameter pore and specific surface area.

SEM micrographs of the surface gelatin obtained in specific condition are shown in Fig. 7. Gelatin showed crack/void-free surface. Smooth and homogenous gelatin surface was obtained from gelatin controlled by some film or surfactant as explained by previously researcher [20-21] However, the less homogeneity and smoothness of gelatin surface might be due to the bother fragmentation on hydrolysis during extraction process. The image of SEM showed that chain morphology of gelatin was changed by transition process to get the loss of molecular order. Although the carbonization of the smooth surface of gelatin more favourable than the crack for carbon production, it is still can be potential source of carbon due to the high carbon content.

The produced carbons in this work are identified as CFG. The BET theory, the basis of the modern IUPAC classification, was used in this study to characterize the nitrogen adsorption isotherms. The analysis of the nitrogen adsorption isotherms shown in Fig. 8 provided an approximate assessment of the pore size distributions on Fig 9. As shown, Fig. 8 had micropore character similar with IUPAC classification these curves resemble the type I isotherm which represents microporous solids having a relatively small external surface area. The porous carbon of gelatin have textural properties with surface area 89 m²/g, pore diameter 15 nm and pore volume 0.01 cm³/g. The results of this study showed that gelatin can be successfully converted into porous carbon with simple preparation. The high textural properties might be obtained with modification of molecular weight of gelatin using variation of temperature, time and concentration solution during extraction process.

IV. Figures and Tables

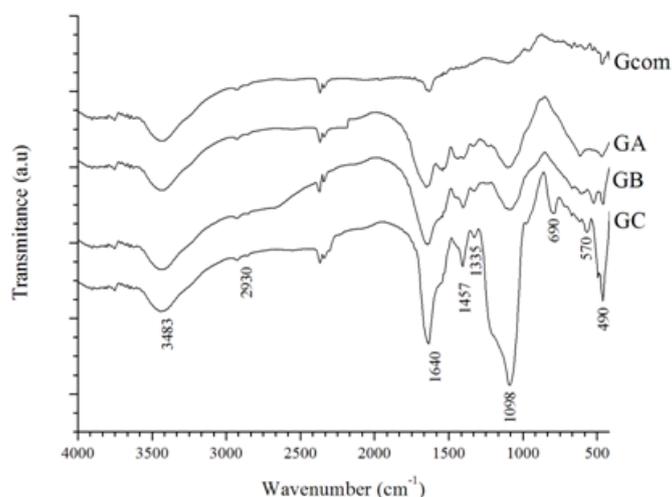


Figure 1. The FTIR spectra for gelatin extracted at different temperature: GA: 45°C; GB. 65°C; GC. 75°C and Gcom. gelatin commercial

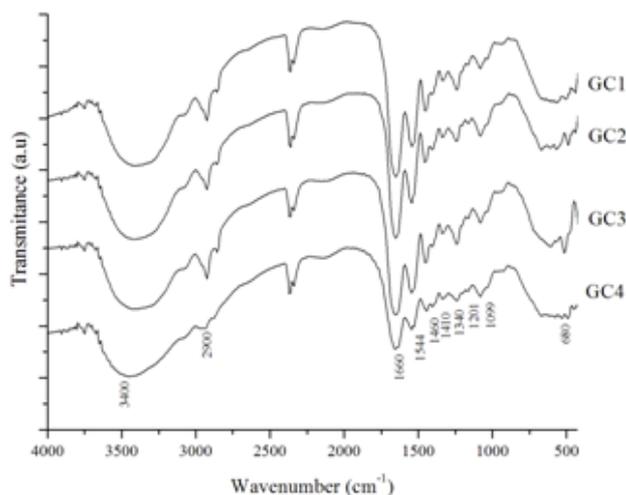


Figure 2. The FTIR spectra for gelatin extracted at 75°C for : GC1. 2h; GC2. 7h; ; GC3. 12h and GC4. 23 h

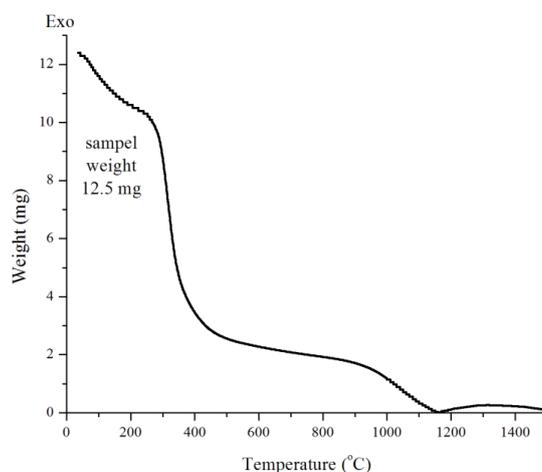


Figure 3. DTA curves for gelatin GC

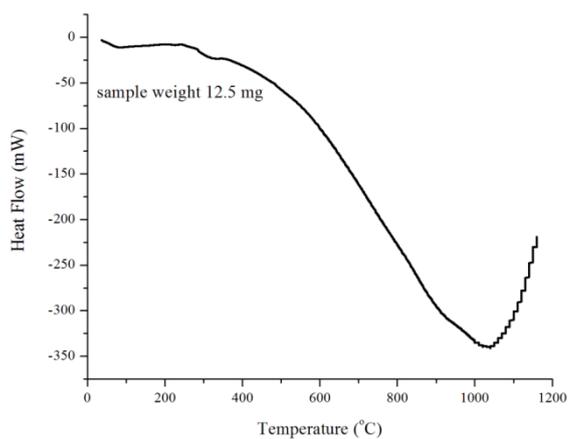


Figure 4.DSC curve for gelatin GC

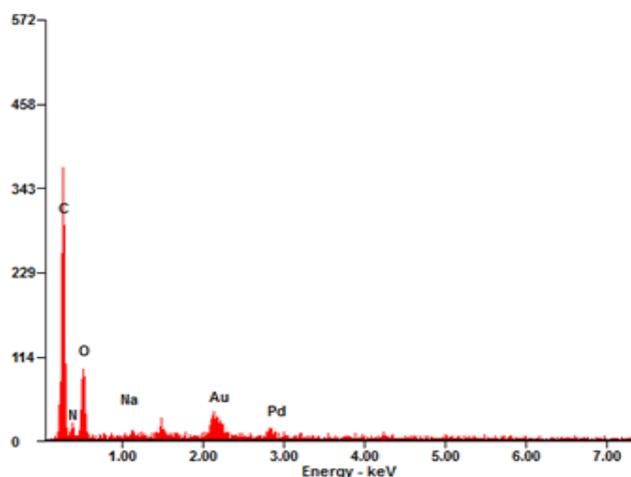


Figure 5. EDAX for elemental analysis for gelatin GC

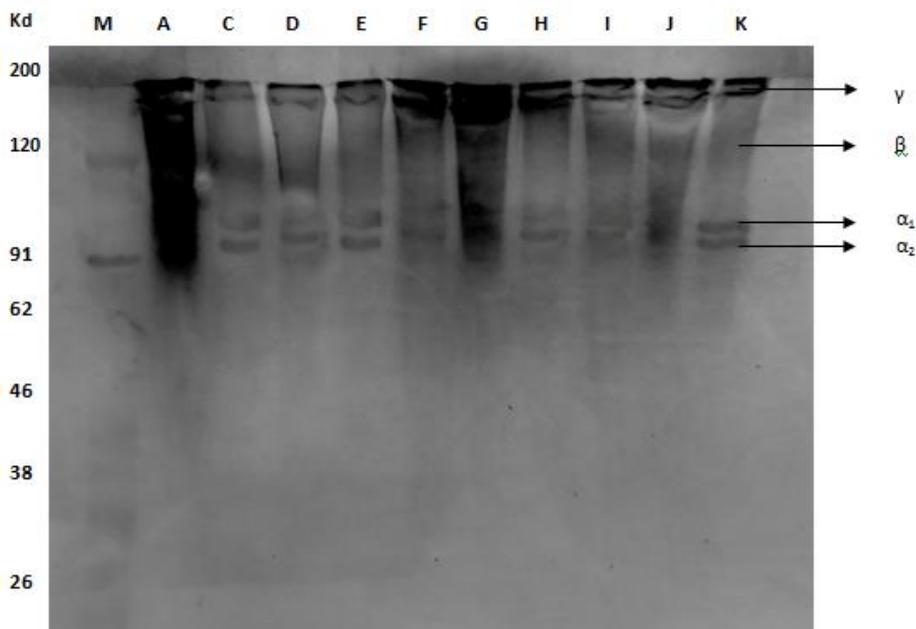


Figure 6. Electrophoretic profile for molecular weight analysis for gelatin GA, GB, GC, GC1,GC2, GC3 and GC4

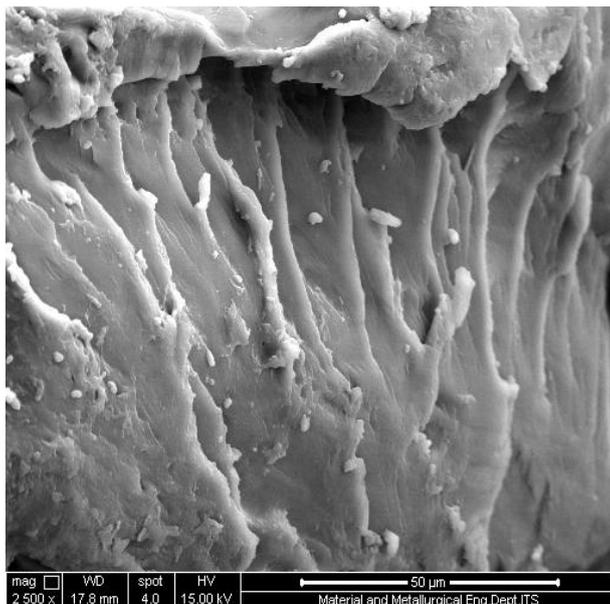


Figure 7. SEM for 3 dimension analysis for gelatin GC

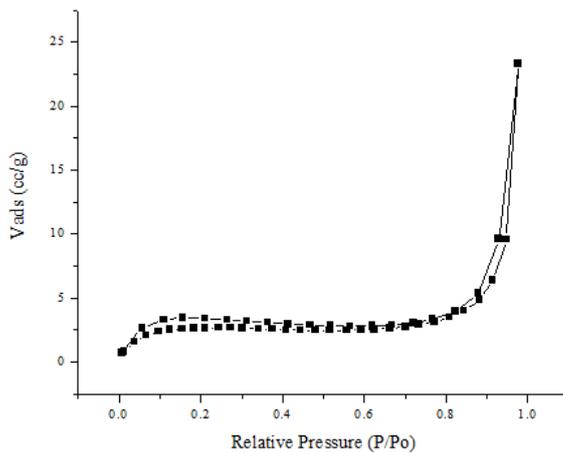


Fig. 8. The Nitrogen adsorption–desorption isotherms for porous carbon from gelatin

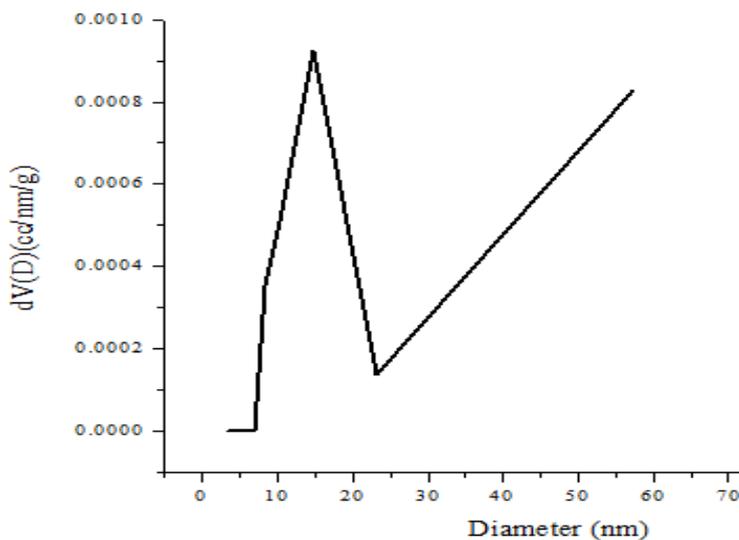


Fig. 9. Pore size distribution for porous carbon from gelatin

V. Conclusion

Gelatin with different characteristics was extracted by the combination of alkali and acid pretreatment. FTIR spectroscopy showed that conversion of collagen to gelatin leads to loss in the triple helical structure and decrease in molecular weight. The secondary structure of gelatin obtained from the cow bone material by acid extractions may vary, with the higher temperature containing more intermolecular associations in the dry state and the low temperature containing more crosslink. The molecular weight range of gelatin extraction was 90-110 kDa with carbon and nitrogen 48% and 15 wt% and thermal stability range is 400-600 °C. Gelatin successfully converted into porous carbon with simple preparation. The porous carbon produced by carbonization of gelatin have surface area 89 m²/g, pore diameter 15 nm and pore volume 0.01 cm³/g.

Acknowledgements

The authors would like greatly to thank for The financial support from the Indonesian Ministry of Education through the Hibah Unggulan Perguruan Tinggi Universitas Gadjah Mada 2015 (Contract number: 232/LPPM/2015).

References

- [1]. S. Kang, J. Jian-chun, and C. Dan-dan, "Preparation of activated carbon with highly developed mesoporous structure from *Camellia oleifera* shell through water vapor gasification and phosphoric acid modification," *Biomass and Bioenergy*, 35(8), 2011, 3643–3647.
- [2]. T. Liou and S. Wu, "Characteristics of microporous / mesoporous carbons prepared from rice husk under base- and acid-treated conditions," *Journal of hazardous materials*, 171, 2009, 693–703.
- [3]. K. Bo, W. Einicke, and O. Klepel, "Templated synthesis of mesoporous carbon from sucrose — the way from the silica pore filling to the carbon material," *Carbon*, 43, 2005, 1918–1925.
- [4]. M. Sobiesiak, "Thermal properties of nanoporous carbons prepared by a template method using different polymeric and organic precursors," *New Carbon Materials*, 27(5), 2012, 337–343.
- [5]. C. Hsu, H. Lin, and C. Tang, "Synthesis of mesoporous silica and mesoporous carbon using gelatin as organic template," *Mesostructured Material*, 2007, 385–388.
- [6]. B. Giménez and M. P. Montero, "Food Hydrocolloids Role of lignosulphonate in properties of fish gelatin films," *Food hydrocolloids*, 27(1), 2012, 60–71.
- [7]. B. Giménez, M. C. Gómez-Guillén, and P. Montero, "The role of salt washing of fish skins in chemical and rheological properties of gelatin extracted," *Food Hydrocolloids*, 19(6), 2005, 951–957.
- [8]. M. C. Gómez-Guillén, B. Giménez, M. E. López-Caballero, and M. P. Montero, "Functional and bioactive properties of collagen and gelatin from alternative sources: A review," *Food Hydrocolloids*, 25(8), 2011, 1813–1827.
- [9]. Z. Zarai, R. Balti, H. Mejdoub, Y. Gargouri, and A. Sayari, "Process for extracting gelatin from marine snail (*Hexaplex trunculus*): Chemical composition and functional properties," *Process Biochemistry*, 47, 12, 2012, 1779–1784.
- [10]. Z. Khiari, D. Rico, A. B. Martin-Diana, and C. Barry-Ryan, "Comparison between gelatines extracted from mackerel and blue whiting bones after different pre-treatments," *Food Chemistry*, 2013, 879–884.
- [11]. M. Ulfa, W. Trisunaryanti, I. I. Falah, and I. Kartini, "Nitrogen Sorption Evaluation of the Porous Carbon Made from Cow Gelatin," *Journal of Chemical Engineering and Chemistry Research*, 1(2), 2014, 101–109.
- [12]. M. Ulfa, W. Trisunaryanti, I. I. Falah, and I. Kartini, "Synthesis of mesoporous carbon using gelatin as source of carbon by hard template technique and its characterizations," *Journal of Applied Chemistry*, 4(5), 2014, 1–7.
- [13]. M. Ulfa, W. Trisunaryanti, I. I. Falah, and I. Kartini, "Studies of kinetic on thermal decomposition of mesoporous carbon of gelatin by thermogravimetric technique," *International Journal of Innovation and Applied Studies*, 7(3), 2014, 849–856.
- [14]. M. Yan, B. Li, X. Zhao, and J. Yi, "Food Hydrocolloids Physicochemical properties of gelatin gels from walleye pollock (*Theragra chalcogramma*) skin cross-linked by gallic acid and rutin," *Food hydrocolloids*, 25(5), 2011, 907–914.
- [15]. H. Aoki, T. Taguchi, H. Saito, H. Kobayashi, K. Kataoka, and J. Tanaka, "Rheological evaluation of gelatin gels prepared with a citric acid derivative as a novel cross-linker," *Materials Science and Engineering: C*, 24(6–8), 2004, 787–790.
- [16]. P. Kittiphattanabawon, S. Benjakul, W. Visessanguan, T. Nagai, and M. Tanaka, "Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*)," *Food Chemistry*, 89(3), 2005, 363–372.
- [17]. Z. Gui-feng, L. I. U. Tao, W. Qian, L. E. I. Jian-du, M. A. Guang-hui, and S. U. Zhi-guo, "Identification of Marker Peptides in Digested Gelatins by High Performance Liquid Chromatography / Mass Spectrometry," *Chinese Journal of Analytical Chemistry*, 36(11), 2008, 1499–1504.
- [18]. G. Mondragon, C. Peña-rodríguez, and A. González, "Bionanocomposites based on gelatin matrix and nanocellulose," *EUROPEAN POLYMER JOURNAL*, 62, 2015, 1–9.
- [19]. M. Bertoldo, F. Cognigni, and S. Bronco, "Preparation of gelatin/polyoxypropylene grafted copolymers by isocyanate promoted 'grafting onto' reaction," *Polymer*, 53(21), 2012, 4595–4603.
- [20]. T. Białopiotrowicz and B. Jan, "Surface Properties of Gelatin Films," *Polymer*, 14, 2002, 9462–9468.
- [21]. S. Iwamoto, M. Nakajima, Y. Kikuchi, and H. Nabetani, "Precision control of the surface structure of gelatin films using micropit plates," *Colloids and surfaces. B, Biointerfaces*, 38, 1–2, 2004, 15–20.