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Physicochemical Properties and Functional Group Assessment of Gelatin Extracted from Cow Bone Bio-Waste for Suitability in Biomedical Application

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Abstract: *The need for biocompatible, bioactive and biodegradable materials which can be utilized as viable biomaterials in Tissue engineering cannot be overemphasized. Cytotoxicity, immunogenicity, non-biocompatibility, non-biodegradability are some service challenges encountered by the use of some biomaterials, however the abundance, bioactivity, non-toxicity and its structural resemblance of native tissue ECM (extracellular matrix) has strategically placed gelatin and gelatin based biocomposites as viable biomaterials for biomedical applications. Gelatin is prepared by thermal denaturation of collagen, from skin and bones of animals generally cows and pigs. In this study gelatin was extracted from bovine bones of original size after cleaning and reduced size of between 0.1-10 mm. The size reduction was done to investigate its effects and variation in basic concentration on the properties of the extracts. Varying molarities of 1, 3 and 5 mol/dm^3 of sodium hydroxide (NaOH) solution were adopted in the pre-treatment process prior to water bath extraction, these treatments were also carried out on unreduced bone samples. Physico-chemical characterisation showed that the Type B gelatin extracts had viscosity values ranging from 3.5 – 4.3 cP, moisture content ranging from 7 – 8%, pH ranging from 4.2 – 5.8 and high protein content ranging from 61 – 64%. The reduction in size of the samples had no significant effect on the yield. Functional group assessment by FTIR confirmed the production of gelatin in all the extracts although with the presence of some inorganic minerals which were present in the raw bone. These properties confirm viability of the extracted gelatin for the production of gelatin-based composites for bone implants in Tissue engineering. Also, the use of cow bone has a positive impact on environmental waste management as the bone waste was converted into a useful product.*

Keywords: *Cow bone bio-waste, gelatin extracts, pre-treatment, NaOH concentration, physico-chemical properties.*

1. INTRODUCTION

The reaction of the body to the presence of implant materials has led to research concerning their suitability in medical procedures. Any implantable material/device intended to work as a body part will definitely be into contact with the tissue of the host. The response of the tissue to the material and the degradation of the material to this new environment are critical considerations in the determination of the materials used in the fabrication of the device [1]. Therefore, there is the crucial requirement for biocompatibility and bioactivity of materials adopted as implants for biomedical and tissue engineering applications. Currently, adoption of biomaterials bridges this gap, a biomaterial is a combination of one or more substances that are derived from synthetic or natural sources, which helps in treatment or replacement of any tissue, organ or function inside the body [2]. Biomaterials create a biomolecular and spatial environment that is needed for cell proliferation and vascularization in Tissue engineering (TE) and Regenerative medicine (RM). The various classes of biomaterials in TE include natural, synthetic and composite biomaterials. Natural biomaterials include protein-based biomaterials (collagen, silk fibroin, gelatin, fibronectin, keratin, fibrin, eggshell membrane) and polysaccharide-based biomaterials (e.g., hyaluronan, cellulose, glucose, alginate, chondroitin, and chitin and its derivative, chitosan), are promising subsets of biomaterials employed in TE because of their bioactivity, biocompatibility, tuneable degradation and mechanical kinetics and their intrinsic structural resemblance of native tissue ECM [3]. These extracellular matrix proteins are widely utilized as bio mimicking materials, as these proteins are the main interactors with cells in vivo and thus are viable materials for use in vitro [4]. They also promote biological recognition which helps cell adhesion, proliferation, cell differentiation and function. Currently the use of gelatin-based biomaterials and composites have gained popularity due to processing techniques arising from recent technological advancement. Gelatin is an animal protein with abundant sources, it is biocompatible, biodegradable, and does not induce antigenicity and toxicity in cells, also gelatin possesses similar structure to collagen

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which is the most abundant ECM protein. Gelatin is prepared by thermal denaturation of collagen, gotten from the skin and bones of animals generally cows and pigs. Gelatin has numerous applications ranging from domestic to industrial purposes. In the past decade, this natural biopolymer has found vast application in bone and cartilage tissue regeneration, cardiac and vascular tissue regeneration, also gelatin-based scaffolds have been tailored to support hepatic tissues. The demand for gelatin has drastically increased over the past decade. The ease of extraction and the countless uses of gelatin have made it a highly sought-after material. It has a wide range of applications in the food industry (emulsifier, gelling agent), cosmetics (components of cosmetic products), pharmaceutical (capsules, ointments), and specialized industries such as cell culture (surface coatings, hydrogels) and regenerative medicine [5].

Chitosan/gelatin composite scaffold with an organized microstructure in consonance with the physiological hepatic tissue was shown to support the growth and proper development of hepatocytes in vitro [6]. Acid demineralization treatment and extraction of gelatin on camel bone waste was conducted in [7], the acidic treatment reduced elemental calcium within five days to yield ossein with collagen-like characteristics. The calcium removal process was engineered by re-organization and morphological modification through removal of hydroxyapatite. Fourier transform infrared (FTIR) spectroscopy analysis revealed presence of nitrogenous organic residue in the final product. Physicochemical properties of gelatin extracted from Buffalo hide pretreated with different acids was investigated in [8]. The study showed that the highest yield of gelatin was obtained by pretreatment with 0.9 M HCl. All types of acid adopted produced gelatin with the physicochemical properties equivalent to commercial bovine gelatin. Pretreatment with 0.6 M citric acid gave the highest viscosity compared to 0.9 M HCl and 1.5 M acetic acid. Thus, the high gel strength and viscosity of the prepared gels recommend buffalo hide as an alternative source of gelatin. Gelatins from black-bone chicken feet (BCFG) and black bone chicken skin (BCSG) were extracted using different NaOH concentrations, and their physicochemical properties were characterized and compared to commercial bovine gelatin (BG). It was found that the yield of BCFG was higher than BCSG however, it contained higher amount of ash. The NaOH concentration did not show strong influence on physicochemical properties of the extracted gelatins. Thermal stability and gel strength of BCSG tended to decrease with increasing of NaOH concentration [9]. The quality of gelatin for a specific application relies to a great extent on its rheological properties. Aside from essential physio-chemical properties, for example, composition parameters, solubility, transparency, appearance, smell, and taste, the principal traits that best characterize the general commercial quality of gelatin are gel strength and thermal stability (gelling and liquefying temperatures). Both gel strength and thermostability, are generally reliant on the molecular properties of gelatin, particularly regarding two fundamental components. The first is the amino acid composition, which is species-specific, and secondly, the sub-atomic weight distribution, which results predominantly from processing conditions [10]. Gelatin based hybrid composites for wound dressing were reviewed in terms of their performance in the treatment of infected, exuding, and bleeding wounds. It was concluded that when gelatin is used in combination with other polymers, it resulted in excellent mechanical properties that are required for ideal wound dressings for biomedical applications [11]. Slaughterhouses produce a tremendous measure of animal bones as waste in their main business. This increasing measure of bone remnants among other waste materials from meat processing industries has been considered as one of the critical environmental challenges faced by the food industry today. An animal would leave about 18 wt. % of bone deposits after it is slaughtered, commercially utilizing these bone residues will impact positively on waste management challenges. Therefore, this study is aimed at extraction, physico-chemical property evaluation and functional group analysis of gelatin extracted from cow bone bio-waste via the base pre-treatment route.

2. METHODOLOGY

2.1 Materials

The materials adopted were cow bone waste obtained from a local slaughterhouse at Olambe junction in Ogun State, Nigeria, sodium hydroxide (NaOH), Hydrochloric acid (HCl) distilled and tap water and Whatman 200mm Filter papers were obtained from the Department of Metallurgical and Materials Engineering Laboratory, University of Lagos, Nigeria. The pieces of equipment are Fourier Infrared Spectrometer (Nicolet iS10, Madison, Wisconsin), Digital Weighing balance, Water bath (DK 420 U-Clear), Digital pH meter (Hanna HI 2210, Woonsocket, RI), Viscometer (Brookfield Engineering, Middleboro, MA), electric oven and a grinding machine.

2.2 Processing and Demineralization of Cow Bone Bio-Waste

The cow bone was weighed using a digital weighing balance, and the weight obtained was 3.25kg. Residual flesh was peeled off of the bones using a knife and blood stains and dirt were rinsed off using tap water. The cleaned bones were boiled in water using an aluminium pot and a gas cooker for 1 hour to remove the fat and oil content in them. This process is called defatting. The defatted bones were dried for seven (7) days under direct sunlight after which the moisture content reduced well enough to permit ease of size reduction. The dried bones were weighed, final weight of 2.20kg was obtained. The bones were split into two samples labelled A and B. Sample A was crushed using a mallet and ground afterwards using a grinding machine to sizes ranging from 0.1 to 10mm. This was done to determine the particle size reduction effects on the pre-treatment and consequently on the extraction process. Sample B contained the dried bones in their original sizes, all samples were stored in separate polythene bags. Sample A was divided into four sub-samples, which were demineralised by soaking in a 2% HCl solution with the bone to acid ratio of 1:2 (w/v) for 24 hours (1 day) to dissolve the mineral matter in the bones, leaving the organic matter. The demineralised bones (ossein) were drained and rinsed with distilled water until the rinse

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water was neutral to litmus. The demineralised bones were then pre-treated for 168 hours (7 days) in separate plastic containers using sodium hydroxide (NaOH) solutions of different molarities with solid to base ratio of 1:2 (w/v). The fourth set of samples was not pretreated with NaOH and was the control and designated as A0 as shown in Table 1. The molarity of the base solutions used for the pre-treatment of each sub-sample is shown in Table 1.

Table 1: Molarities of the NaOH solutions used for the pre-treatment of sample A

Sample	**Weight (g)**	**Molarity of NaOH (mol/dm^3)**
A0	250	0
A1	250	1
A2	250	3
A3	250	5

Figure 1: Pre-treatment process for Sample A

Sample B which was in the original size was divided into four sub samples: B0, B1, B2 and B3 as shown in Table 2, B0 did not undergo pretreatment, B1, B2 and B3 sub-samples went through the same demineralization and pre-treatment process as described for sample A. The molarity of the base solutions used for the pre-treatment of each sub-sample is shown in Table 2.

Table 2: Molarities of the base solutions used for the pre-treatment of sample B

Sample	**Weight (g)**	**Molarity of NaOH (mol/dm^3)**
B0	**150**	**0**
B1	150	1
B2	150	3
B3	150	5

Figure 2: Pre-treatment process for Sample B

2.3 Extraction of Gelatin

Samples A1, A2 and A3 (now ossein) were drained and rinsed with tap water until the rinse water was almost completely neutral to litmus. The ossein were placed in beakers of distilled water (1:2 w/v), and the extraction was carried out at 80°C for 3 hours using a water bath. The extraction process was also carried out for sample A0 which was not pre-treated. The solutions after extraction were filtered afterward using filter papers and stored in separate plastic containers. Each filtrate was labelled according to the sample from which they were extracted as shown in Table 3.

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Table 3: Designations of extracted Gelatin filtrates from sample A

Sample	Filtrate
A0	GA0
A1	GA1
A2	GA2
A3	GA3

Figure 3: Extraction process for samples in the water bath.

Extraction was carried out on B0, B1, B2 and B3 according to the method described for sample A. The solutions were filtered afterward using filter papers and stored in separate plastic containers. Each filtrate was labelled according to the sample from which they were extracted as shown in Table 4.

Table 4: Sample designations for extracted gelatin filtrates from sample B

Sample	Filtrate
B0	GB0
B1	GB1
B2	GB2
B3	GB3

2.4 Drying and Yield of Extracted Gelatin Samples

The filtrates were placed in labelled beakers and placed in the oven at a temperature of 110℃ for 20 hours until the gelatin samples were dried. The dried samples were weighed and the yield for all the extracts were calculated using the equation below:

$$\%Yield = \frac{weight\ of\ dried\ gelatin}{weight\ of\ processed\ cow\ bones} \times 100 \quad (1)$$

2.5 Physico-Chemical Properties and Functional Group Evaluation of Extracted Gelatin Samples

The viscosity (cP) of the gelatin solutions (6.67%) was determined at 30±0.5°C using a Brookfield digital viscometer (Brookfield Engineering, Middleboro, MA) equipped with a no. 1 spindle at 60 rpm. In the pH determination, distilled water (10ml) was added to 1g of each of the samples and the solutions were heated at 45°C for 5 minutes to dissolve the gelatin powder. The solutions were cooled to room temperature and the pH values were determined during magnetic stirring using a pH-meter Hanna HI 2210, Woonsocket, RI, calibrated against pH 4 and pH 7 buffers [12]. The colour of the gelatin extracts was macroscopically examined using the eye and recorded. In the odour analysis, solutions of 6.67% were prepared by placing 0.5g gelatin samples and 7 ml distilled water into test tubes. The tubes were capped and then placed into a 50°C water bath to dissolve. Afterwards, the test tubes were covered with aluminium foils. The odour specifications were evaluated organoleptically using a 6-point scale (0: no odour, 1: very light, can be sensed when carefully evaluated, 2: mild, easily detectable, 3: strong but not offensive, 4: strong and offensive, 5: very strong and offensive) after which the mean and standard deviation values were recorded for 3 results for each sample. The moisture content was determined gravimetrically after oven drying the samples at 105°C until they reached a constant weight. The ash content was determined gravimetrically by burning the samples at 550°C until they acquired a grey-white ash colour.

The lipid content of the samples was determined by Soxhlet extraction method [13]. The protein content of the samples was determined by Kjeldahl method with 5.4 as the protein conversion factor for gelatin extract. The conversion factor of 5.4 was used because collagen, the main protein in cow bones, contains a nitrogen ratio of 18.7%. All analysis were carried out in triplicate, after which the average was used.

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2.6 Functional Group Evaluation

The functional groups present in the ossein (collagen samples prior to extraction) and gelatin samples were analyzed using Nicolet iS10 Fourier Transform Infrared (FTIR) Spectroscope, 2 mg of the samples were mixed with 100 mg of potassium bromide (KBr) and placed on the crystal cell of the FTIR spectrometer. The samples were analyzed for different functional groups in the region of 4400 – 350 cm-1 at room temperature. The flow chart for the methodology of gelatin extraction adopted is shown in Figure 4.

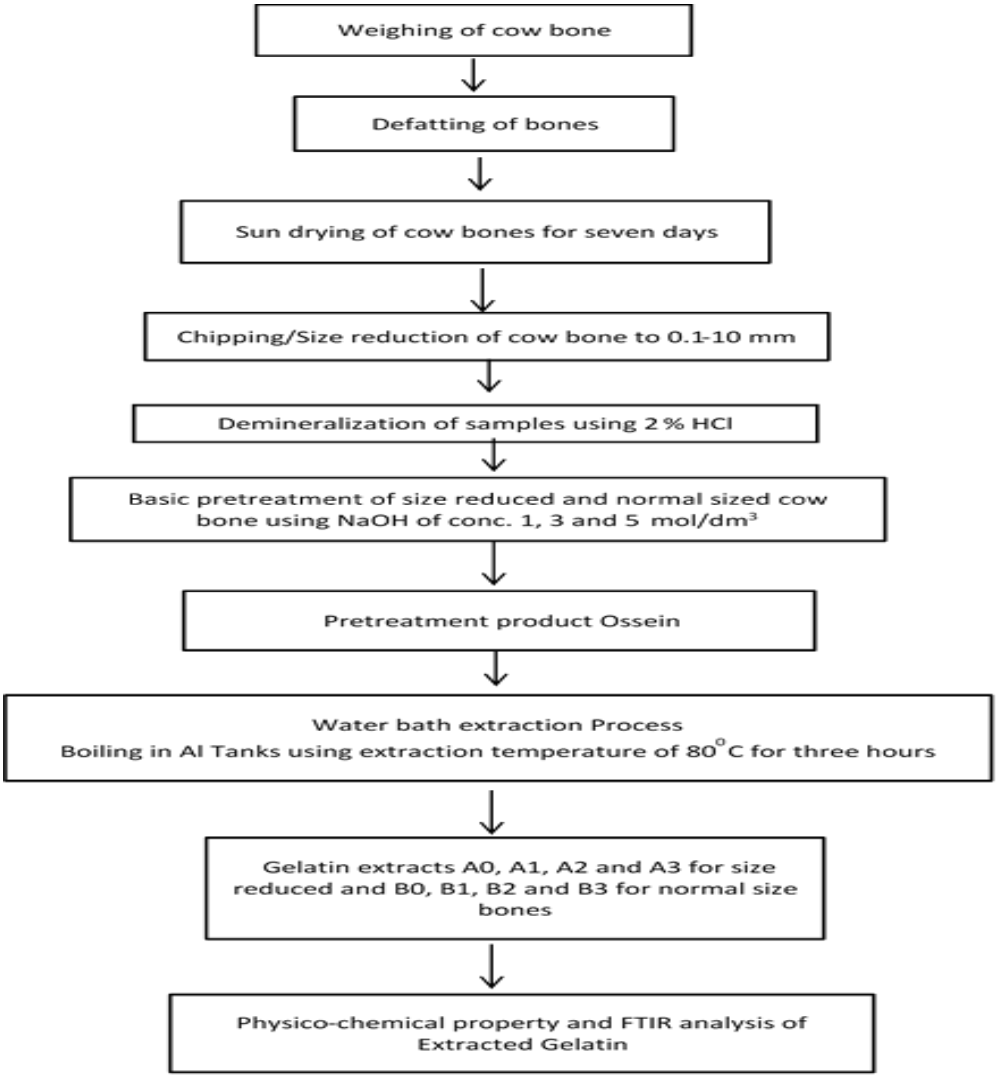

Figure 4: The flow chart for the methodology adopted for Gelatin Extraction.

3. RESULTS AND DISCUSSION

3.1 Extracted Gelatin Samples

The packaged and labelled samples of gelatin that were extracted by adopting the various routes and dried are shown in Figure 5 (a and b) for samples A and B. It is important to mention that for samples A0 and B0 that were not pre-treated, it was observed that no hydrolytic breakdown occurred during the water bath extraction as there was no physical change observed, the bone samples remained the same throughout water bath treatment. This shows that pre-treatment step is necessary to introduce structural changes to enable the production of water-soluble collagen necessary for gelatin extraction [14].

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Figure 5: The packaged and labelled dried samples of gelatin.from A and B cowbone

3.2 Yield of the Extraction Process

The yield of the extracted gelatin from bone samples A and B, were calculated and tabulated as shown in Tables 5 and 6. The chart for the yield versus the concentration of the sodium hydroxide (NaOH) adopted for the pre-treatment is also shown in Figure 6 for the ground and unground cow bone waste. The results as seen in Figure 6 show that the reduction in particle size of the bovine bone had no significant effect on the yield of the extracted gelatin as the highest yield observed was from the original bone samples B. Pre-treatment is targeted at breaking down the minerals like calcium and phosphate to release collagen for gelatin extraction. The base concentrations adopted here were sufficient to enable this reaction occur both in the reduced and original bone samples, hence the insignificant effect on the yield. The yield was directly proportional to the molarity of the solution used for the pre-treatment of the bovine bones of reduced particle size, however there was an inverse relationship between the yield and the molarity of the solution used for the pre-treatment of the bovine bones of original size. A higher yield is obtained from bovine bones of original size at a low pre-treatment base molarity of 1 mol/dm^3 (Table 6) and from bovine bones of reduced size at a high base molarity of 5 mol/dm^3 (Table 5). The reduction in the surface area aided the pre-treatment reaction in the reduced size bone samples. There was no yield for the GA0 sample as no gelatin was extracted in the water bath extraction process indicating that the cross-linkages between polypeptide chains in the cow bone raw material need to be broken down during basic pretreatment, a crucial process that aids breakdown of fibrous structure of collagen to yield gelatin [15].

Table 5: Yield of gelatin extracted from bone sample A

Sample Name	Sample Weight (g)	Raw Material Weight (g)	Molarity for pre-treatment (mol/dm^3)	%Yield	%Yield
GA0	0	100	0	$=\frac{0}{100}\times 100$	0%
GA1	8	250	1	$=\frac{8}{250}\times 100$	3.2%
GA2	11	250	3	$=\frac{11}{250}\times 100$	4.4%
GA3	13	250	5	$=\frac{13}{250}\times 100$	5.2%

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Table 6: Yield of gelatin extracted from bone sample B

Sample Name	Sample Weight (g)	Raw Material Weight (g)	Molarity for pre-treatment (mol/dm^3)	%Yield	%Yield
GB0	0	800	0	0	0
GB1	12	150	1	$= \frac{12}{150} \times 100$	8%
GB2	8	150	3	$= \frac{8}{150} \times 100$	5.33%
GB3	6	150	5	$= \frac{6}{150} \times 100$	4%

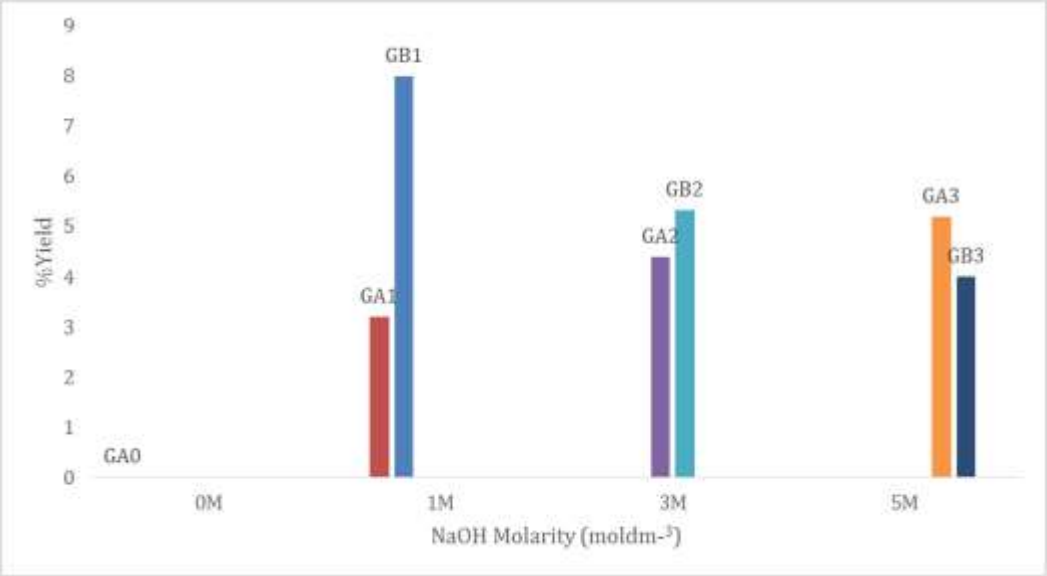

Figure 6: Chart of yield versus NaOH molarity for size reduced and original sample

3.3 Viscosity of the Extracted Gelatin Samples

The plots for the viscosity of the extracted gelatin samples with NaOH molarity are shown in Figure 7 for reduced and original size samples respectively. Gelatin extracted from bovine bone material of original size i.e., B samples have a higher viscosity compared to the gelatin extracted from bovine bone material of reduced size. This can be attributed to surface area increase of the reduced samples which enabled more effective pre-treatment reaction. This caused breaking of bonds in the bone structure, which led to molecular weight reduction. This reduced molecular weight gave rise to the lower viscosities of the reduced size bone samples. Highest viscosities were obtained from bovine bone of reduced and original sizes respectively with NaOH base of molarity 3 mol/dm^3. These deductions are supported by the viscosities of the extracted gelatins in [16], however in [17], higher NaOH molarity gave gelatin of low viscosities. It is necessary to mention at this juncture that since there was no yield from the non-pre-treated sample GA0 and GB0, no further analysis was carried out.

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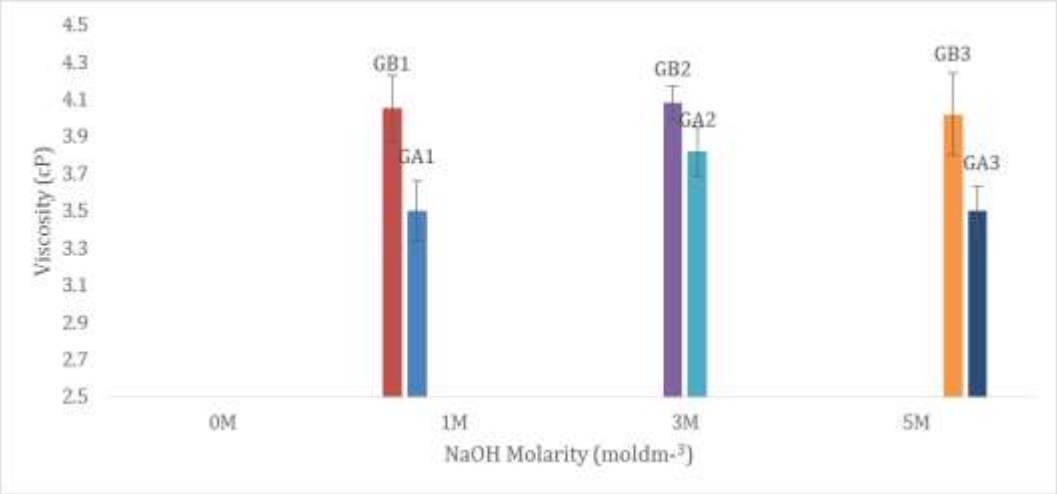

Figure 7: Chart of viscosity versus NaOH molarity of extracted gelatin

3.4 pH of the Extracted Gelatin Samples

The plot of pH versus NaOH molarity is shown in Figure 8 for the gelatin extracts from the reduced and original size cow bone. The pH value range for all the extracts (4.24-5.29) indicates their category as type B gelatin. The range of pH values of B extracts (5.29-5.4) met the requirement of values as stipulated in the standard recommendation for gelatin used in numerous applications including medical applications by the Gelatin Manufacturers Institute of America (GMIA 2012). The increase in molarity of NaOH used for the pretreatment process had a proportionate increase in the pH of the gelatin extracts for the reduced particle sized bone samples. However, this trend was not observed for the original sized bone, where the lower molarity of 1 mol/dm^3 gave the highest pH of 5.47. The pH influences the gel strength of the extracted gelatin as shown in the study by [12].

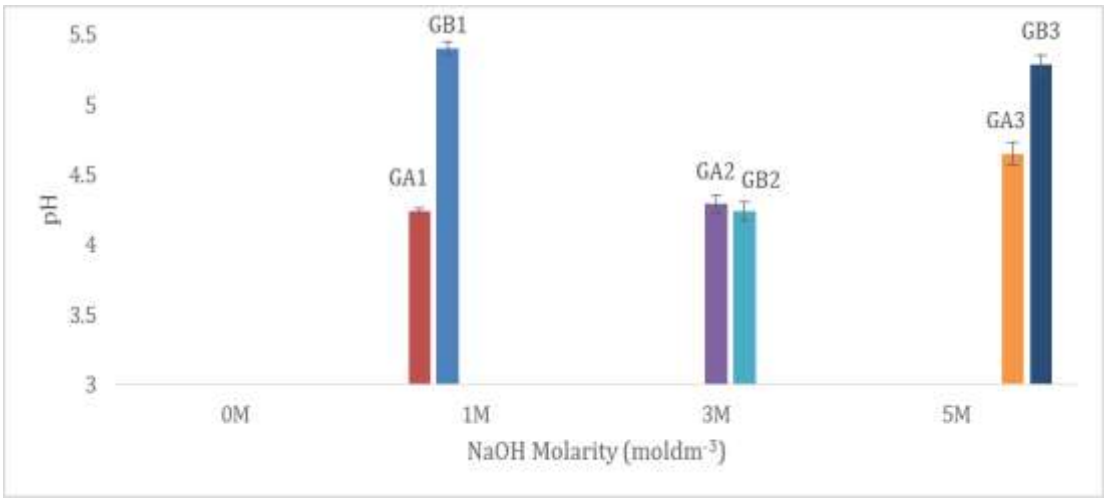

Figure 8: Chart of pH versus NaOH molarity of extracted gelatin

3.5 Colour of Extracted Gelatin Samples

The increasing molarity of the base used for pre-treatment did not significantly have effect on the colour variation of GA and GB extracts. This could be as a result of the extraction time which was the same for all the gelatins. The colour of the extracts ranged from brown, light brown to white as shown in the Table 7 below. It was observed that the colour did not affect the functional properties of gelatin.

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Table 7: Color of extracted gelatin with concentration of NaOH adopted.

S/N	Sample	Molarity (M)	Colour after pre-treatment	Colour of extract
1	GA1	1	Grey	Brown
2	GA2	3	Grey	Brown
3	GA3	5	Grey	Light brown
4	GB1	1	Brown	White
5	GB2	3	Light Brown	Light Brown
6	GB3	5	Light Brown	White

3.6 Odour of extracted gelatin samples

The results for the odour analysis are shown in Table 8 for size reduced and normal bovine samples respectively. The analysis was done in triplicate for each sample and the mean and standard deviation values were recorded as shown in the tables. It was observed that the gelatin extracted from A samples have a mild odour that can be easily detected. The intensity of this odour decreased slightly with an increase in the NaOH molarity used for pre-treatment. While the gelatins extracted from B samples have a strong but non-offensive odour which also appeared to decrease with increasing NaOH molarity used for pre-treatment. The results are consistent with the values observed for commercial bovine gelatin in the study conducted by [18].

Table 8: The Odour values of extracted gelatin samples

Sample Name	**Molarity for pre-treatment (mol/dm^3)**	**Odour**
GA1	1	1.96±0.06
GA2	3	1.98±0.08
GA3	5	1.86±0.06
GB1	1	2.98±0.30
GB2	3	2.96±0.33
GB3	5	2.68±0.25

3.7 Proximate Analysis of the Extracted Gelatin Samples

The results for the proximate analysis of the extracted gelatins are shown in Figures 9-12 for moisture, protein, ash and lipid contents respectively. Also, three results were taken for each sample and the mean and standard deviation values were recorded, the results show that gelatins extracted from B had a higher moisture, lipid and ash content but the gelatins extracted from A had a generally higher protein content.

Generally, the range of results for the moisture contents (Figure 9) of all the extracts were between 7.10 – 8.20%. These fall within the range of values of 6.56 – 12.70% recorded for the extracted gelatins in the study conducted by [19]. The moisture contents of the samples extracted from B are consistent with the standard recorded by the Gelatin Manufacturers Institute of America, 2012. Those of sample A are in consonance with the result from [20]. According to the findings of this study, the reduction in size of the raw material caused a reduction of the moisture content of the extracts.

The standard range for the protein content in commercial gelatin as stipulated by GMIA. (2012) is 85-92%, which is higher than the range of results gotten for this study (61.9-64.67%) as shown in Figure 10. In this study increasing the molarity of the pre-treatment solution did not have a significant effect on the protein content but generally the reduced sample A showed increased protein content in the extract than the sample B. This can be attributed to the increased surface area for the pre-treatment acid to react.

In this study, the reduction in the size of the raw material reduced the ash content of the extracted gelatins as compared to the unreduced size bone samples (Figure 11). The increasing molarity increased the ash content in gelatins extracted from the reduced A samples, whereas this was the reverse for the unreduced B samples. The ash content values are consistent with the result recorded in the study of [18] and Gelatin Manufacturers Institute of America. (2012).

The lipid contents recorded in this study as shown in Figure 12 (1.56-2.16%) are higher compared to the results of [19] which are 0.01-0.22% and 0.45% in [18] for commercial gelatin. According to [19] the lipid content of gelatin is only affected by the extraction temperature. Higher temperature leads to the extrusion of lipid from the bone marrow. In this study, the reduction in the size of the bones led to a reduction in the amount of lipids in the extracted gelatin compared to the extract from the raw material of original size.

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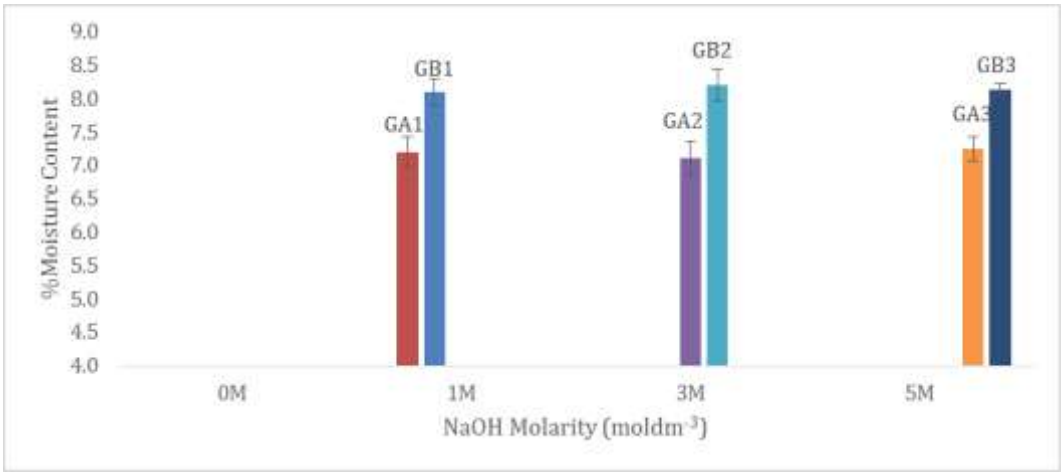

Figure 9: Chart of % moisture content versus NaOH molarity

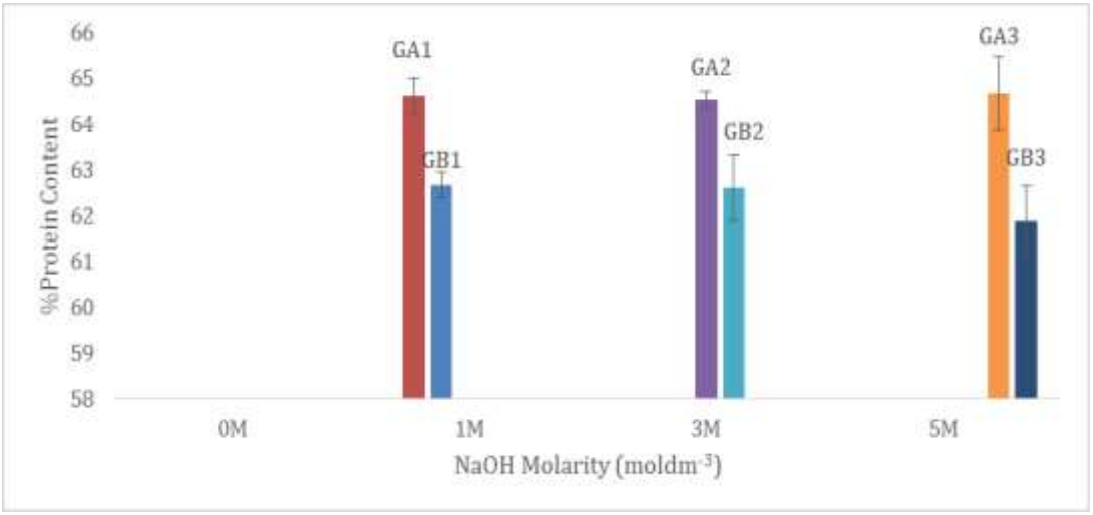

Figure 10: Chart of % protein content versus NaOH molarity

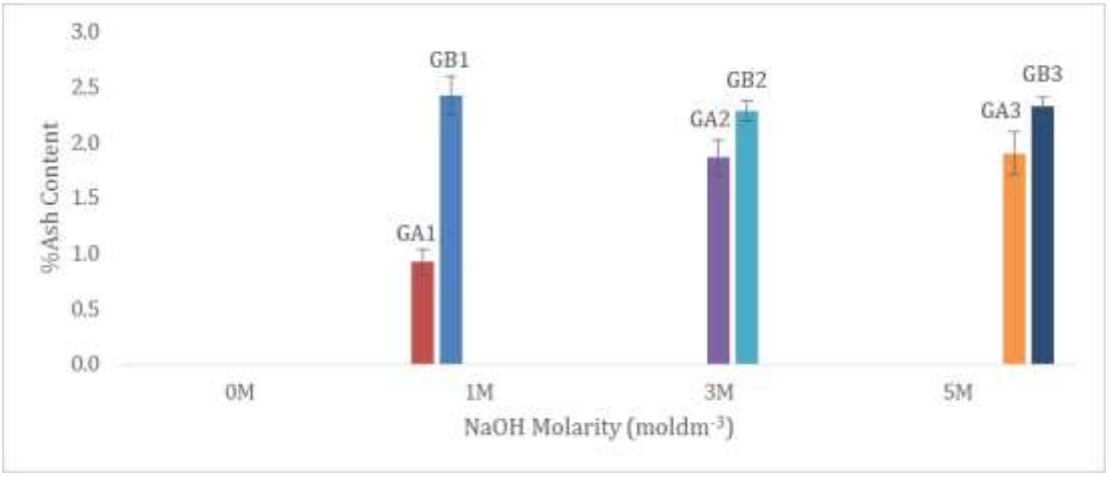

Figure 11: Chart of % ash content versus NaOH molarity

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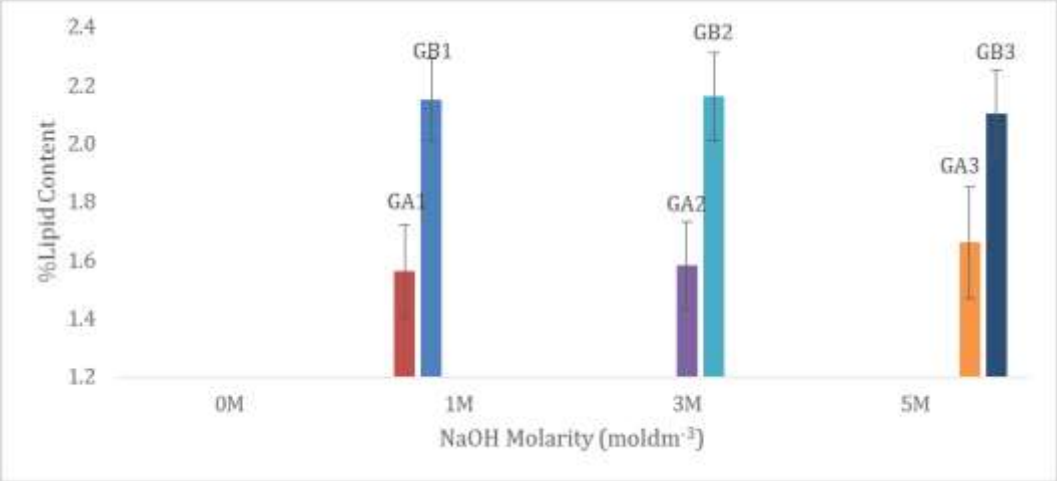

Figure 12: Chart of % Lipid content versus NaOH molarity

3.8 Fourier Transform Infrared (FTIR) Spectroscopy

This is usually carried out to determine the functional groups and secondary structures of extracted gelatin. This is particularly important in the production of gelatin-based composites which serve as biomaterials in RM and TE. It helps determine potential reinforcing compounds and also possible chemical reactions towards adequate cross linking and production of composites with good mechanical properties. In this study, the band shape, wave number at the band, intensity and chart shape were used to characterize the differences in the structure of the gelatin extracts. Typically, gelatin contains functional groups found in proteins such as hydroxyl (O-H), carbonyl (C=O), and amine (N-H) groups. Proteins comprise of amino acids joined together by amide bonds, gelatin being a derived protein has absorption bands in the amide band region of the FTIR spectra; amide-I represents C=O stretching/hydrogen bonding coupled with COO, amide-II represents bending vibration of N-H groups and stretching vibrations of C-N groups between wavelengths 1200-1550 cm^{-1}, amide-III is related to the vibrations in plane of C-N and N-H groups of bound amides found between wavelengths 900-1200 cm^{-1} [21] and [22]. Two other regions found in gelatin spectra are the amide A and B regions (4000-2000 cm^{-1}). The most important region to examine the secondary structures of gelatin is the amide-I region between wavenumbers 1700-1600 cm^{-1} [23]. The FTIR analysis of ossein (pre-treatment products using 1, 3 and 5 mol/dm^3 of NaOH for size reduced bone) and gelatin extracts from A and B samples are shown in Figures 12, 13 and 14 respectively.

3.9 Ossein (Pre-treatment product)

The peaks for 1 M NaOH pretreated ossein (Figure 13) occurred at 1047 cm^{-1} ;1367-1310 cm^{-1}; 1417 cm^{-1}; 1686 cm^{-1}; 2966 cm^{-1}; and 3430 cm^{-1} indicating phosphate ion, amide III; C-N stretch, N-H bend and C-H groups; Amide I disordered structure without hydrogen bond; stretching C-H bands and N-H stretching bands respectively. The spectrum for the cow bone coincided with that of 1 M NaOH pretreated ossein. The spectrum for 3M NaOH pre-treated ossein showed noticeable peaks at 1047 cm^{-1}; 1245 cm^{-1}; 1644 cm^{-1}; 2929 cm^{-1}; and 3436 cm^{-1} indicating the presence of symmetric PO^{2-}; Phosphate I (stretching PO^{2-} symmetric vibration); Amide I; C-H, Lipid region, CH_3, CH_2 -Lipid and protein, C-H stretching vibrations of methyl (CH_3) and methylene (CH_2) groups and olefins, OH stretching; OH asymmetric stretch respectively. The spectrum for 5 M NaOH pre-treated ossein showed peaks at 760 cm^{-1}; 1089 cm^{-1}; and 2929 cm^{-1} indicating out of plane bending vibrations; stretching PO^{2-} symmetric, Phosphate I (stretching PO^{2-} symmetric vibration); and C-H, Lipid region, CH_3, CH_2 -Lipid and protein, C-H stretching vibrations of methyl (CH_3) and methylene (CH_2) groups and olefins, OH stretching bands respectively, these groups are in consonance with other studies in literature [7, 23]. It is also important to mention at this juncture that the peaks in the spectrum for the cow bone coincided with that found in the spectrum for 1 M ossein. This could be attributed to the concentration of the base solution adopted, as it was low and inadequate for proper demineralization to occur. The presence of phosphate ion in all the ossein samples shows that the demineralization carried out using HCl acid and the pre-treatment base concentration were inadequate to remove the phosphate compounds in the bone.

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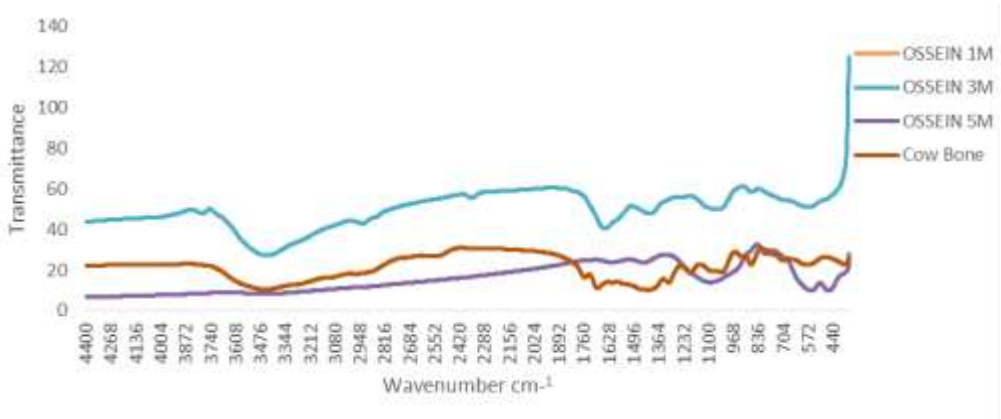

Figure 13: FTIR Spectra for Ossein samples

3.10 GA Samples

The peaks for GA1 (Figure 14) were displayed at 1047 cm^{-1}; 1195 cm^{-1}; 1641 cm^{-1}; 3424 cm^{-1} indicating the presence of symmetric PO^{2}- stretching bands; Amide III band region; Amide I; and stretching O-H asymmetric respectively. GA2 showed peaks at 3426 cm^{-1}; 1644 cm^{-1}; 1199 cm^{-1} ;1050 cm^{-1}, indicating the presence of stretching O-H asymmetric; Amide I; Collagen; and CO-O-C, C-O stretching coupled with C-O bending of the C-OH of carbohydrates, Glycogen, Ring stretching vibrations mixed strongly with C-H in plane bending, Protein amide I absorption, C-OH bonds, C-O stretching (carbohydrates), CH in plane bending vibrations, the aromatic CH bending and rocking vibrations, region of phosphate vibrations, carbohydrate residues attached to collagen and amide III vibrations (in collagen). GA3 had peaks at 759 cm^{-1}; 918 cm^{-1}; 1025-1105 cm^{-1}; 1637 cm^{-1}; 2938 cm^{-1}; 3456 cm^{-1} indicating the presence of out of plane bending vibrations; phosphodiester; C-H, Lipid region, CH_3, CH_2 -Lipid and protein, C-H stretching vibrations of methyl (CH_3) and methylene (CH_2) groups and olefins, OH stretching; Cholesterol, stretching vibrations of CH_2 and CH_3 of phospholipids, cholesterol and creatine. These functional groups are consistent with those in [21, 22] and [24, 25]. Thus, the extracted samples contain functional groups such as C-N, N-H, C-H and OH as exists in the structure of gelatin.

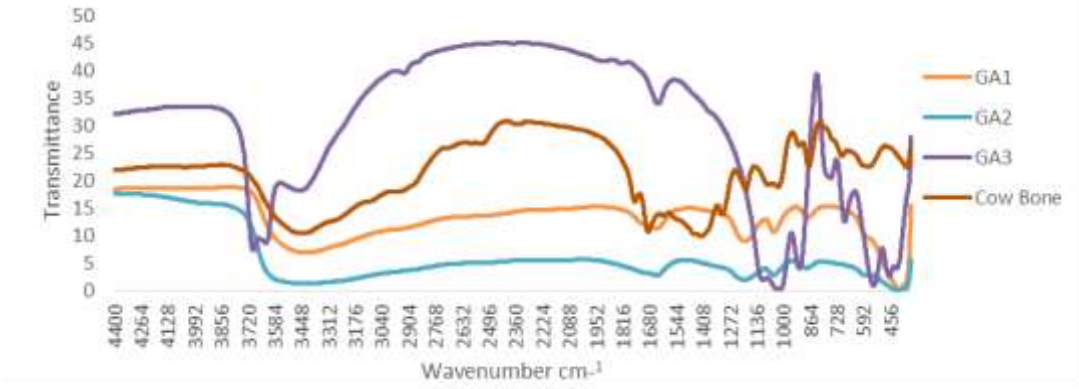

Figure 14: FTIR Spectra for gelatin samples from A

3.11 GB Samples

The peaks in the spectra of GC1 coincided with that of cow bone (Figure 15) at 13067-1310 cm^{-1}; 1417 cm^{-1}; 1686 cm^{-1}; 2966 cm^{-1}; and 3430 cm^{-1} which indicated Amide III; stretching C-N, deformation N-H and deformation C-H groups consistent with [7]; Amide I disordered structure without hydrogen bond consistent with [23, 26]; stretching C-H bands and; N-H stretching bands respectively. GC2 showed peaks at 1639 cm^{-1} indicating Amide I region and H-O-H deformation of water. Other noticeable peaks in the spectrum are between 2928-2930 cm^{-1} indicating stretch bands of C-H and 3458 cm^{-1} indicating OH bonds, GC3 showed peaks at 3471 cm^{-1} and 3419 cm^{-1}; 2929 cm^{-1}; 1628 cm^{-1}; 1383 cm^{-1}; 1316 cm^{-1};1258 cm^{-1}; 1079 cm^{-1}; 1026 cm^{-1}; 903 cm^{-1}; 603 cm^{-1} indicating OH bonds; C-H, Lipid region, CH_3, CH_2 -Lipid and protein, C-H stretching vibrations of methyl (CH_3) and methylene (CH_2) groups and olefins, OH stretching bands; Amide I; stretching C-O, deformation C-H, deformation N-H; Amide III band components of proteins, collagen; Amide III, PO_2^- asymmetric; PO_2^-; CH_2OH groups; Phosphodiesters; CH out of plane bending vibrations and ring deformation of phenyl respectively. These functional groups are in consonance with those in [21, 22].

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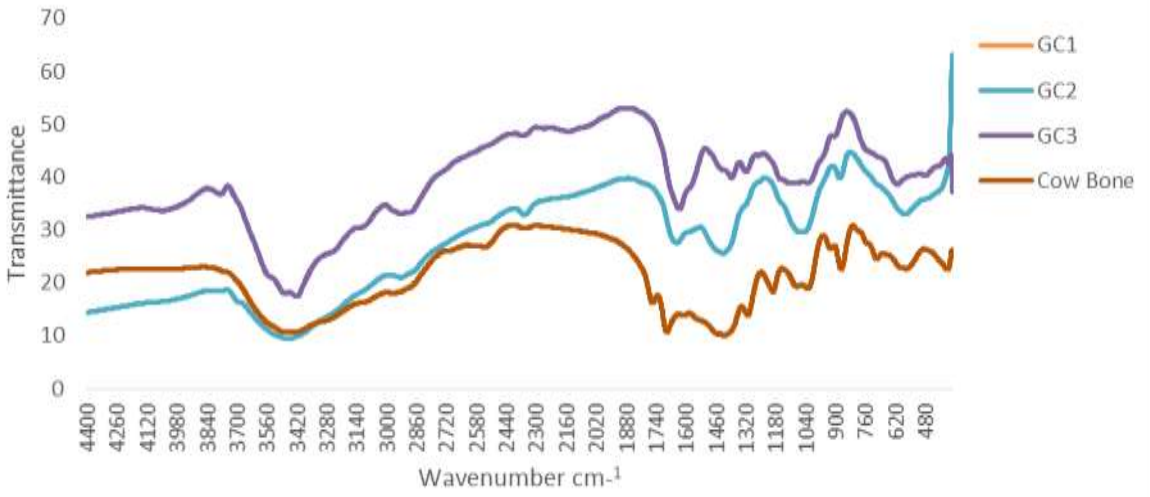

Figure 15: FTIR Spectra for gelatin samples from B

4. CONCLUSION

In this study, Type B gelatin was successfully extracted from bovine of reduced and original sizes, the reduction in particle size was done to determine if physico-chemical properties would be impacted. The gelatin's extracted had physicochemical properties: viscosity, pH, moisture content and protein content, similar to commercial gelatin and consistent with results from other publications [21-26]. High protein content was observed in the extracts, this contributed to their high viscosities. Also, reduction in bone particle size had no significant effect on the yield of the extracts, however it affected the viscosity of the extracts. The FTIR analysis showed the existence C-N, N-H, C-H, O-H, Amide I and Amide III functional groups which are characteristic peaks in the structure of gelatin. In the manufacture of gelatin-based biomaterials and composites in regenerative medicine (RM) and tissue engineering (TE), these groups act as potential reinforcing sites by initiating chemical reactions towards adequate cross linking and production of composites with good mechanical properties. However, the presence of inorganic functional groups was observed, an indication that the demineralization treatment was not effective. This could be attributable to the concentration of NaOH solution adopted for the pre-treatment. It is hereby recommended that further studies could be carried using higher concentration of the NaOH solution and also allowing a longer period of time in the water bath than the three (3) hours done in this study. Also, higher temperatures exceeding 80 ^{o}C can be investigated. Hence, this study revealed the potential of cow bone as a readily available raw material for production of gelatin for gelatin-based biocomposites in biomedical application.

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