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EFFECTS OF ACID TREATMENT ON THE FABRICATION OF PARTIALLY DEACETYLATED CHITIN NANOFIBERS

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EFFECTS OF ACID TREATMENT ON THE FABRICATION OF PARTIALLY DEACETYLATED CHITIN NANOFIBERS

THESIS WORK

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DECLARATION

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G.M. Ahshanuzzaman

Dedicated

To My

Beloved Parents

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ABSTRACT

Partially deacetylated chitin nanofibers were extracted from shrimp shells by mechanical treatment (high speed blender at 37000 rpm) under acetic acid, ascorbic acid, lactic acid and maleic acid condition to compare the effects of acids on nanofibers. The obtained nanofibers under these four types of acid conditions were different in their characteristics such as viscosity, dewatering time, microscopic image and SEM image. The nano-sheets prepared from ascorbic and maleic acid treated nanofibers were very smoother and had a fine nanofibers network with uniform fibers than acetic and lactic acid treated nanofibers. Furthermore, the optically transparent nano-composites were obtained by impregnation of all four types of nano-sheets into neat acrylic resin matrix. The nano-composites under acetic acid and ascorbic acid conditions showed the MOR value of 13.7 MPa and 13.3 MPa respectively and the MOE values were 1.67 GPa and 1.63 Gpa respectively. The light transmittance of lactic and maleic acid treated nanocomposites were both 83%, and the ascorbic and acetic acid condition were 81% and 77% respectively. It is conceived that this variation in characteristics of nanofibers due to the different acid treatments will lead to a momentous step in advancing the field of nanotechnology.

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1. Introduction

1.1 Background and significance

Nanotechnology is the wonderful gift of modern science which is very blessing for us to enrich our life and efficiency. It offers the understanding and control of matter at dimensions between approximately 1 and 100 nanometers with fundamentally superior properties and functionalities because of their minute structure (Roco, 2003). For this the researchers are giving special attention on nanotechnology and nanofibers. Nanofibers have been used as reinforcement in the composite materials and able to increase the mechanical performance with higher surface area and the number of defects might be reduced at the nano level (Yano *et al.*, 2005). Though the nanofibers have been extracted from both synthetic and natural sources, the researchers are now rushing to extract nanofibers from natural polymers over the synthetic polymers such as cellulose or chitin because of high crystallinity, biodegradability, biocompatibility, renewability and readily availability (Biswas *et al.*, 2013).

Chitin is the second most abundant biopolymer after cellulose, and is the main component of the exoskeletons of insects and crustaceans such as crabs and shrimps (Qi *et al.*, 2013). Because these raw materials are produced in large quantity as food waste every year, effective utilization of chitin is of high significance for development of a sustainable society (Qi *et al.*, 2013). Chitin consists of numerous crystalline microfibrils in living bodies which are now convertible to nanofibrous elements having different morphologies (Ifuku and Saimoto, 2012). When moderate deacetylation is applied to a-chitin, N-acetyl groups present on the surface of crystalline alpha-chitin microfibrils are selectively removed and converted to C_2-amine groups (Qi *et al.*, 2013). It can be characterized by degree of deacetylation (DDA), molecular weight and their distribution, residue protein etc (Jana *et al.*, 2013).

The degree of deacetylation influences the physical, chemical and biological properties of chitosan, such as acid base and electrostatic characteristics, biodegradability, self-aggregation, properties (Hussain *et al.*, 2013). The degree of deacetylation allows one to define the terms chitin and chitosan, that is, chitosan is usually defined as the derivative that is soluble in dilute acidic solutions (Balázs and Sipos, 2007). The lowest DDA corresponding to chitosan varies in literature and ranges from 40% to 60% (Hussain *et al.*, 2013). But in this situation the chitosan exhibits a crystalline structure that is not used in many other applications. That is why The crab chitin was first treated with 33% (w/w) NaOH to partially remove the acetyl groups on the C-2 position (Wang *et al.*, 2016).

The 33% (w/w) NaOH deacetylation is very convenient because on this limit of deacetylation the chitin reaches a condition that is neither fully chitosan nor fully chitin, the both qualities of chitin and chitosan are preserved in this state. And the further nanofibrillation process is also influenced by various acids during the preparation of nanofibers from those partially deacetylated chitin. This is also very important in this regard. Under acidic conditions, protonation of these amine groups brings about positive surface charges, leading to formation of electrostatically repulsive forces between the microfibrils in water (Qi *et al.*, 2013). Mild mechanical treatment of surface-protonated a-chitin microfibrils under acidic conditions triggers their ultimate nanofibrillation or individualization (Qi *et al.*, 2013).

Chitin nanofibrils with ultimately small lateral dimension and high degree of nanofibrillation can be obtained without major damage to the original crystallinity using this method (Fan *et al.*, 2010). In many biomedical, catalytic, and adsorptive applications, the degree of nanofibrillation or individualization, surface area, and surface charge of crystalline polysaccharide nanofibrils can make a great difference (Jayakumar *et al.*, 2010). Different acids have their different effects on the various aspects of nanofibers. By observing the effects of acids on nanofibrillation and other qualities of nanofibers, a new experiment is designed to evaluate the effects of various acids on the characteristics of nanofibers in this study. Four types of acids are used in this study, they are acetic acid, ascorbic acid, lactic acid and maleic acid.

1.2 Objectives

- To extract and characterize partially deacetylated chitin nanofibers from shrimp shells by adding different acids through a simple mechanical process.
- To fabricate transparent nanocomposites and to evaluate the effect of acid treatments on transmittance and mechanical strength of chitin nanocomposite.

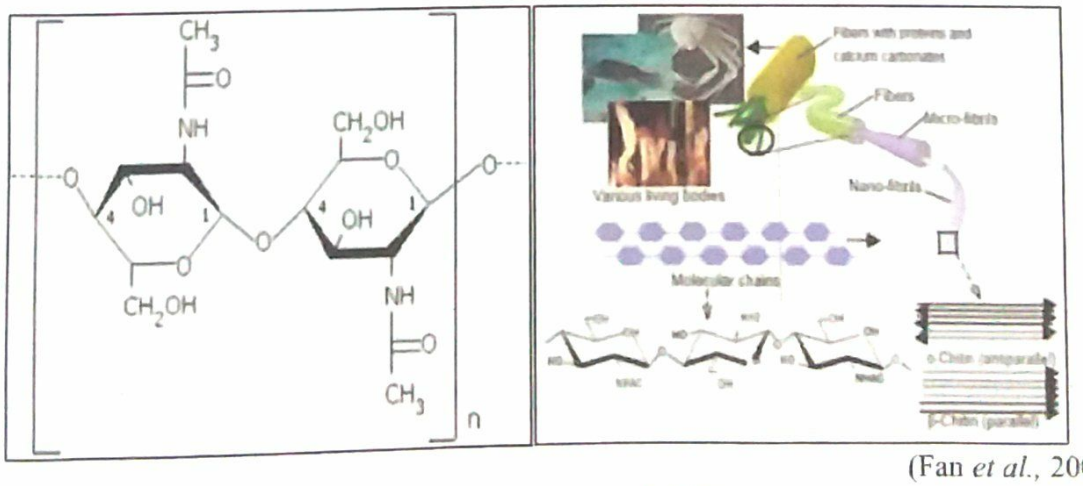
2. Literature review

2.1 Nanotechnology and nanofibers

Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers. Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering. Nanotechnology is the engineering of functional systems at the molecular scale (Nogi *et al.*, 2006). This covers both current work and concepts that are more advanced. In its original sense, nanotechnology refers to the projected ability to construct items from the bottom up, using techniques and tools being developed today to make complete, high performance products. Nanofibers as having a diameter of less than one micron, although the National Science Foundation USA, (2005) defines nanofibers as having at least one dimension of 100 nanometer or less. Nanofibers are an exciting new class of material used for several value added applications such as medical, filtration, barrier, wipes, personal care, composite, garments, insulation, and energy storage.

2.2 Chitin, its structure and properties

Chitin is a polymer formed primarily of repeating of β (1-4) 2acetamido 2-deoxy-D-glucose or N-acetyl glucosamine (Nogi *et al.*, 2006). Chitin is a nitrogenous polysaccharide, which is white, hard, inelastic, found in the outer skeleton of insects, crab, shrimp and lobsters and in the internal structures of other invertebrates (Fan *et al.*, 2008). Its structure is similar to the structure of cellulose, except that acetyl amino groups have replaced the hydroxyl groups in position-2. Native chitin has a limited application potential, but the deacetylated chitin has a wide spectrum of applications ranging from large scale technical applications.

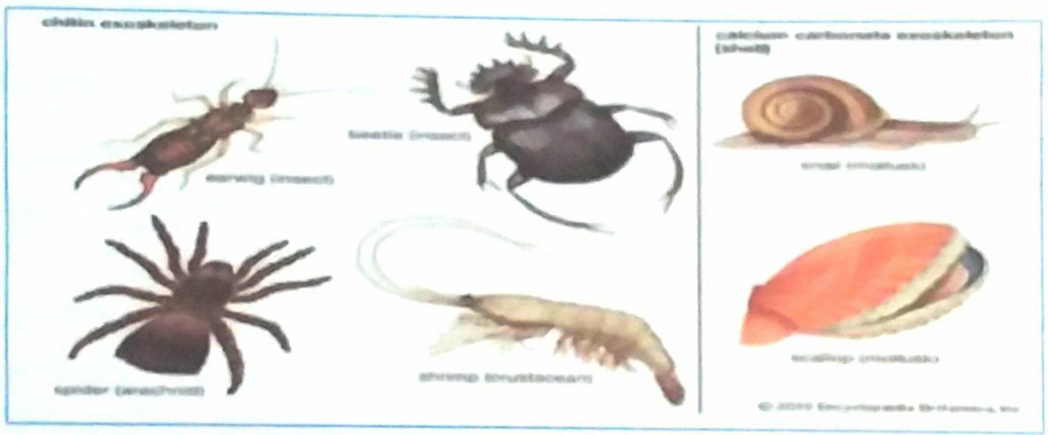


(Fan *et al.*, 2008)

Figure 2.1: Structure of chitin

2.3 Sources of chitin

Chitin was found in animals and plant sources. The major sources of chitin are shrimps, crabs, squilla, lobsters, krill, clams, oysters, squid, insects and fungi. Allan *et al*., (1981) estimated the chitin content of selected crustacean, insects, molluscan organs and fungi. Though the main source of chitin is animals, it is frequently present as a cell wall material in plants, replacing cellulose or sometimes occurring together with cellulose. Chitin is extensively produced from fungi varieties such as *Aspergillus niger*, *Mueor rouxii*, *Peneeillium notatum* (Tan *et al*., 1996; Knorr, 1984). Chitin is present in marine diatoms, protozoa and the cell walls of several fungal species.



(Knorr, 1984)

Figure 2.2: Sources of chitin

2.3.1 Shrimp shells - a potential source of chitin

In the processing of shrimps for human consumption, between 40 and 50 % of the total mass is waste (Percot *et al*., 2003). About 40 % of the waste is chitin, incrusted with calcium carbonate and astaxanthin, and containing meat and a small amount of lipid residues (Sajomsang *et al*., 2009). A small part of the waste is dried and used as chicken feed, while the rest is dumped into the sea, is source of chitin.

Table 2.1: Components (%) of shrimp shells

Composition	Dry Weight %
Chitin	30-40%
Protein	35%
$CaCO_3$	30%
Lipids	5-10%

(Sajomsang *et al*., 2009)

2.4 Extraction of chitin

At first the raw shrimp shells are air-dried for 3 days. As the shrimp shell chitin is intimately associated with proteins, lipids, calcium carbonate and pigments, the dried shell is first treated with hydrochloric acid (HCl) to remove calcium carbonate (Abe *et al.*, 2007). Dried shrimp shell is treated with 7% HCl solution for 24 hours at room temperature (22 ^{0}C) to remove the mineral salts. After thorough washing with abundance of water, the treated sample is dispersed in 4% sodium hydroxide (NaOH) solution and boiled at 60 ^{0}C for 4 hours under vigorous stirring to remove proteins (Khorrami *et al.*, 2012). The treatment is repeated four times in order to complete removal of residual proteins. The shrimp shell is turned into soft and white chitin-only shell (Illum, 1998).

2.5 Deacetylation and partial deacetylation of chitin

The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a compound (chitosan) with a high degree chemical reactive amino group (-NH2). The N-deacetylation of chitin is either performed heterogeneously, or homogeneously. Commonly, in the heterogeneous method, chitin is treated with a hot concentrated solution of NaOH during few hours, and chitosan is produced as an insoluble residue deacetylated up to ~85%–99%. According to the homogeneous method, alkali chitin is prepared after dispersion of chitin in concentrated NaOH (30 g NaOH/45 g H2O/ 3 g Chitin) at 25 °C for 3 h or more, followed by dissolution in crushed ice around 0 °C. The acetamide group of chitin was converted into an amino group by deacetylation, and the electrically charged amino group on the chitin surface accelerated disintegration due to electrostatic repulsion. That is, α-chitin was partially deacetylated by 33% NaOH treatment. The degree of deacetylation was approximately 30%.

2.6 Nanofibrillation of chitin

The method of preparing chitin NF from crab shell is applicable to a variety of prawn shells, since prawn shell is also made up of a hierarchical organized structure. Three types of prawn shells were chosen as starting materials: Penaeus monodon (black tiger prawn), Marsupenaeus japonicas (Japanese tiger prawn), and Pandalus eous Makarov (Alaskan pink shrimp) (*Ifuku et al.*, 2011). These species are widely cultivated around the world as important food sources, although their shells are often thrown away as food industrial waste. After proteins and minerals were removed, purified wet chitins extracted from the shells were disintegrated using a grinder. A uniform structure of the chitin NFs was observed. The fiber structure was very

similar to that of NFs obtained from crab shells. Since the prawn exoskeleton is made up of a finer structure than crab shell, the mechanical disintegration of prawn shell was easier than that of crab shell (Chen *et al*., 2008).

2.7 Applications of chitin, chitosan and their nanofibers/nano-composites

Chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability, adsorption, and ability to form films, and to chelate metal ions (Rout *et al*., 2001). Chitosan is useful in a wide application in various industries such as pharmaceuticals, biochemistry, biotechnology, cosmetic, biomedical, paper industry, food and textile industries and others (Muzzarelli, 1985) The importance of chitosan nanofibers and composite are mentioned in the Table 2.2

Table 2.2: Usefulness of chitosan and chitin-chitosan nanofibers

Application area	Specific use
Wastewater treatment	• removal/recovery of metal ions from wastewaters, copper, chromium • removal and binding of dyes • biological denitrification
Food	• food and nutrition • bioconversion for the production of value-added food products • preservation of food • filtration and clarification of fruit juices
Biomedicine	• burn and wound dressings for humans and animals • antitumour activity • immunostimulating properties in mammals and plants • antiviral and anti-Candida albicans activities
Agriculture	• plant elicitor • stimulation of chitinase and glucanase production (increased response to pathogen attack)
Textile and paper	• textile fibres • paper manufacture (additive)
Biotechnology	• chitin affinity chromatography to selectively adsorb chitinase from a fermentation broth • enzyme and whole cell immobilizer

(Muzzarelli, 1985)

3. Materials and methods

3.1 Materials

Fresh shrimp shells of tiger shrimp (*Peneus monodon*) were collected from Jemini Sea Foods Limited; Rupsha, Khulna; Bangladesh. The shrimp shells were combines of body and head shells. In this experiment, body shells were used to prepare partially deacetylated chitin nanofibers. For separation of proteins, minerals and deacetylation, NaOH (99.9%) and HCl (37%) were used. Four types of acids such as acetic acid (99%), ascorbic acid L(+), lactic acid (90%) and maleic acid (99%) are used to prepare four types of nanofibers sample.

3.2 Preparation of partially deacetylated chitin nanofibers and nano-sheets

3.2.1 Chitin preparation

The chitin of shrimp shells combined with proteins, lipids, calcium carbonate and pigments. Shrimp shells were washed thoroughly in water to remove adhering sand, dirt etc. The shrimp shells were washed several times then dried at room temperature and then grounded by normal grounder to make smaller particles about 3 to 5 mm in size. Then 100gm of dried grounded shrimp shells were prepared for next chemical treatments.

Demineralization- The samples obtained were demineralized using 10% HCl solution; 900 mg water and 100 mg HCl for 24 hours with continuing magnetic stirring and then carefully washed at 4-6 hours with continuous water flow to remove mineral salts. This process was repeated for two times for better removing of minerals from the shells.

Deproteinization- A solution of 6% NaOH which contains 60gm NaOH and 940gm of water was prepared. Then the demineralized shells were mixed with the NaOH solution in a kettle and the kettle was put into a water bath and boiled at 60o C for 8 hours then carefully washed at 4-6 hours with continuous water flow to remove proteins. This process was repeated for two times for better removing of proteins from the shells.

Decoloration- It is the process by which the pigments are removed. The chemically processed demineralized and deproteinized shells were then added with 200gm of ethanol in a kettle with continuing magnetic stirring kept at about 24 hours at room temperature. After 24 hours the sample was carefully washed at 4-6 hours with continuous water flow to remove coloring agents or pigment from the sample. This process was repeated for two times to extract best quality chitin. Then the chitin was obtained and the yield was measured as 18.68%.

3.2.2 Partially deacetylated chitin preparation

The chitin obtained were then deacetylated for the removal of acetyl groups from chitin through the process of deacetylation. The purified chitin were put into a beaker with 33% NaOH solution with chitin to NaOH solution ratio of 1:25 (gmL^{-1}), in a water bath at 60°C for 6 hours. After that a condition of deacetylated chitin was evolved. In this condition this partially deacetylated chitin washed with water and dried room temperature overnight.

3.2.3 Partially deacetylated chitin nanofibers preparation

The deacetylated chitin samples were dispersed in water with a concentration of 1 wt% to form a slurry. Several drops of acetic acid were added to the slurry to adjust the pH value to 3–4 to facilitate fibrillation and to generate electrostatic repulsion force among the fibers for the best blending operation. It is the general concept of electrostatic theory, the two like-charged particles tend to repel each other by generating repulsive forces, to facilitate fibrillation. The suspension was then placed in a high-speed blender (Vita-Mix Blender, Osaka Chem. Co. Ltd.) and blended for 10 minutes at a rotating speed of 37000 rpm and was kept in a wet condition. The same process was also done with the addition of another three types of acids such as ascorbic acid, lactic acid and maleic acid in deacetylated chitin sample during blending operation to produce nanofiber of ascorbic acid, lactic acid and maleic acid condition respectively. The four types of sample were then kept in several beakers at wet condition for further experiment.

3.2.3.1 Characterization of partially deacetylated chitin nanofibers

Viscosity

Viscosity is tested is to determine the degree of influence individual factors such as polymer concentration, percent solvent, solvent type, and the molecular weight of the polymer have on the overall viscosity of the of the solution. Viscosity of those partially deacetylated chitin nanofibers sample were tested by VTE-03 viscometer using number 3 rotor. Four suspensions of 460gm of chitin slurry were prepared based on different acid conditions.

Microscopic image observation

For obtaining the microscopic view of partially deacetylated chitin nanofibers sheets they were coated with Cedar-wood Essential Oil and carefully observed the fiber orientation of the nanofibers. Several photographs of microscopic image were taken for experiment.

3.2.4 Partially deacetylated chitin nano-sheet preparation

The 1 wt% chitin nanofiber sample of four types were dispersed in water to make 0.1 wt% solution. From that 1 wt% nanofiber sample, 5gm sample was taken and mixed with water for making of 0.1 wt% solution to make a nano-sheet. Then this 0.1 wt% was taken in a beaker and kept on magnetic stirrer for about 1-2 hour to fibrillate the fibers. Then the following steps were done for making the nano-sheets for other four types condition.

Dewatering and vacuum filtration- The suspensions were vacuum-filtered using filter papers (pore size: 0.3 μm) for removing water from the 0.1 wt% solution. Then gradually after several minutes the water was removed from the solution by vacuum-filtration. It took 9 minutes to 16minutes average for the all four types acid condition for completely dewatering.

Wet sheet formation- Then after dewatering and vacuum-filtration the wet thin sheets were separated as a mat from the filter paper very carefully and kept on mesh. Then the wet sheet was kept sandwiched between two mesh for the next step of hot pressing.

Hot pressing- The wet sheet with mesh then instantly taken for hot pressing. During hot pressing 1Mpa pressure and 110^0C temperature was maintained for 10 minutes for making nano-sheets. After 10 minutes the sheet was taken out and the partially deacetylated chitin nano-sheets were ready for final analysis. The whole process was repeated to prepare four types of acid condition nano-sheet.

3.2.5 Optically transparent film preparation

The partially deacetylated chitin nano-sheets were impregnated into neat acrylic resin matrix to produce optically transparent nanocomposites. Then the new things of optical transparent partially deacetylated chitin nanocomposites were prepared.

3.2.5.1 Characterization of nano-sheet and optically transparent nano-film

Dewatering time

During vacuum filtration the water was removed from the solution of 0.1 wt% partially deacetylated chitin nanofibers solution, this process is called dewatering. The dewatering time of each of the sample during sheet making was measured by simple measurement of minutes. It was measured carefully for the all acid condition samples.

Field-emission scanning electron microscopic (FE-SEM) image observation

The all four types partially deacetylated chitin nanofiber sheets were coated with an approximately 2-nm layer of platinum by an ion sputter coater (JFC-1600, JEOL Ltd.) and observed with a field emission scanning electron microscope (JSM-6700F, JEOL Ltd).

Light transmittance

The light transmittances of partially deacetylated chitin nano-sheets were measured using a UV-visible spectrometer (U-4100, Hitachi High-Tech. Corp.) with an integrating sphere 60 mm in diameter at wavelengths from 200 to 800 nm. Regular light transmittance was measured by placing the specimens 25 cm from the entrance port of the integrating sphere. The total light transmittance of the samples were also measured by passing light into various positions of the sheets and calculated an average.

Tensile strength test

The tensile properties were measured using an Instron 3365 universal testing machine. The specimen gage lengths were measured with a caliper for each sample upon gripping, and the crosshead speed was set at 1mm/min. the specimens were 35mm long, 5mm wide and 40-60 micro meter thick.

4. Results and discussion

4.1 Effect of different acid treatments on partially deacetylated chitin nanofibers

4.1.1 Effect of acid treatments on viscosity

Figure 4.1 shows the amount of viscosity of four types of acid conditions nanofibers. It is seen that the viscosity of maleic acid treated nanofibers is 82 mPa.s and the viscosity of other three types of nanofibers such as acetic acid, ascorbic acid and lactic acids are 85, 86 and 89 mPa.s respectfully. All four types of nanofibers are very viscous more or less equally. But the lactic acid treated nanofibers are more viscous among them. This is due to the effective repulsive force that facilitates the nanofibrillation process. And the anionically-charged and dissociated second carboxyl group in the aspartic acid molecule at pH 3.5 may have been shielded by this amine group through the formation of an intra-molecular ionic bond (Qi et al., 2013).

On the other hand the acetic and ascorbic acid treated nanofibers are about to same viscosity as shown in this figure. Ascorbic acid known as vitamin C, behaves as a vinyl carboxylic acid and forms two major resonance structures when dissociated (Qi et al., 2013). As the acetic acid concentration increased from 10% to 90%, the viscosity increased as the solution became increasingly viscous (Ifuku *et al.*, 2007). Fan et al. (2008) reported that the higher viscosity might be due to individualization of partially deacetylated α-chitin fibrils at higher level in acidic water. Qi *et al.*, (2013) also reported that, the frequency of cross-linkages formed between cationic PDACh fibrils by the polyvalent conjugated bases probably increased with the amount of additional dissociated carboxylic groups on each acid molecule of ascorbic acid. For this reasons, viscosity varies with different acids treatment.

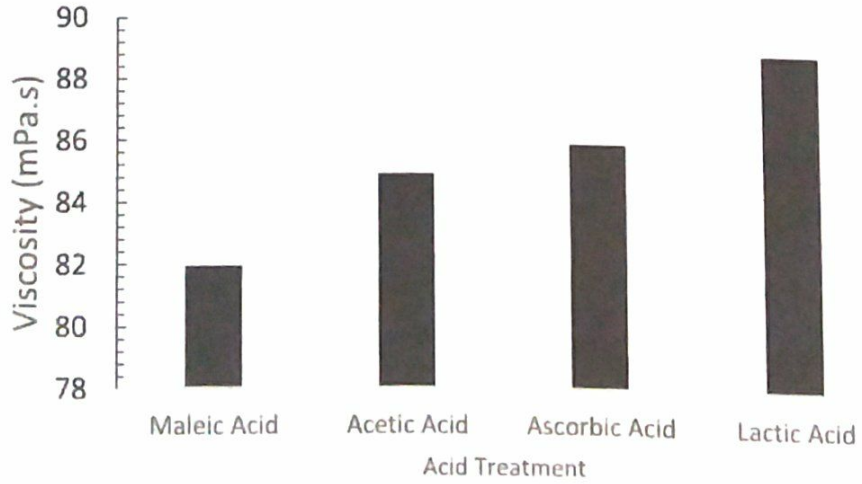


Figure 4.1 Viscosity of nanofibers

4.1.2 Effect of acid treatments on microscopic image

Figure 4.2 shows the effect of acid treatments on nanofibers. It shows that no big particles are seen in the nanofiber slurry, resulting in a much more homogeneous nanofiber structure in all the four types of nanofibers. This figure represents that the effectiveness of acids on nanofibers during the fibrillation procedure. This is due to the presence of the positive charges on the surface of the nanofibers, which results from the protonization of the amino groups of chitin in acidic conditions (Shams *et al.*, 2011). When crab shell chitin was mechanically disintegrated in water by high speed blender, chitin nanofibers with variable widths are obtained which indicates that removal of matrix followed by simple mechanical treatment is not sufficient for fibrillating the individualized chitin nanofibers from shrimp shells (Biswas *et al.*, 2013). On the other hands the electrostatic repulsive force produced by surface protonation was the most vital condition to fibrillate individualized chitin nanofibers (Fan *et al.*, 2008). Accumulation of fibers were not occurred here that is why there are no fiber bundles seen in it. Fibers were evenly distributed, that is why contact surface is higher in all four types of acid condition nanofibers.

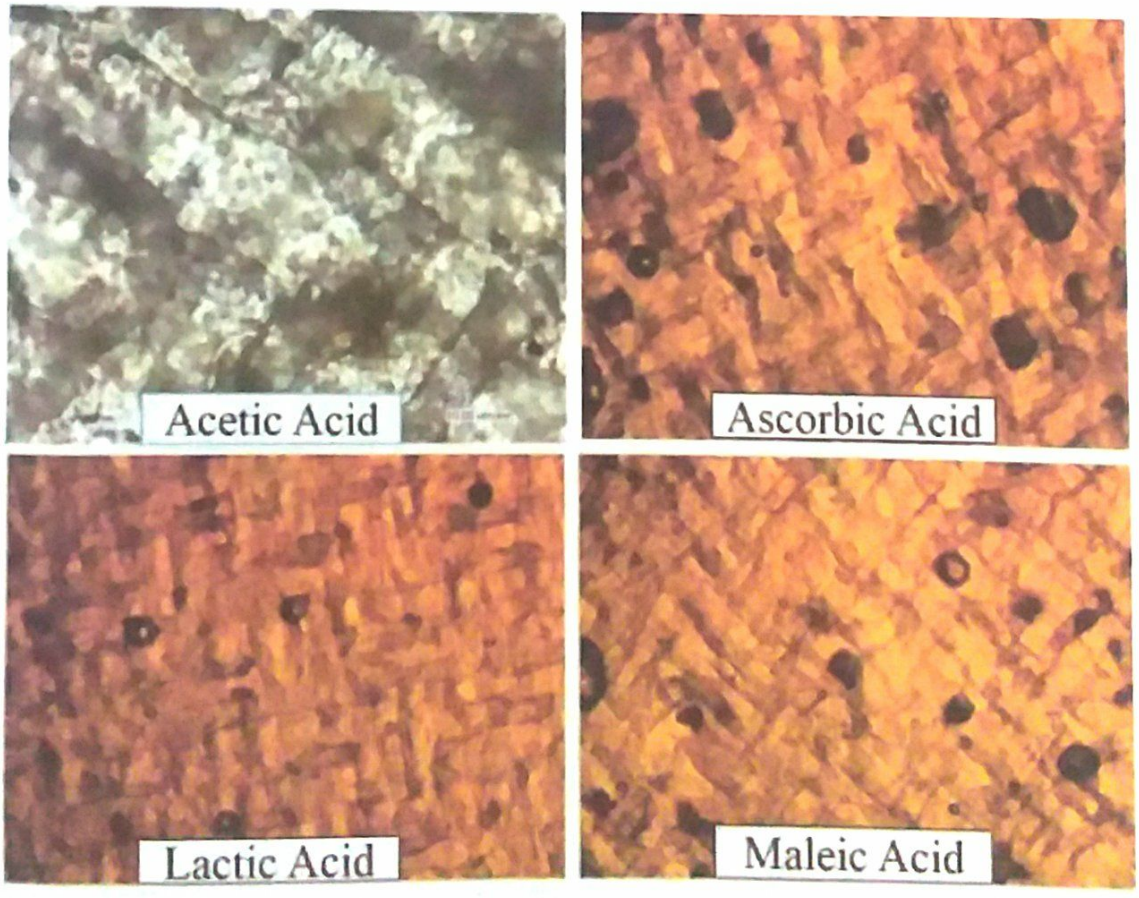


Figure 4.2 Microscopic image of the nanofibers

4.1.3 Effect of acid treatments on FE-SEM image

Figure 4.3 shows the FE-SEM images of all four types of sheet. It represents that clear uniform web-like structure is seen in the ascorbic acid treated nano-sheet. This is due to the proper removal of pigments and others matrixes and the effective electrostatic repulsion forces created by ascorbic acid during nanofibrillation process. The anionically-charged and dissociated second carboxyl group in the ascorbic acid molecule at pH 3.5 may have been shielded by this amine group through the formation of an intra-molecular ionic bond, causing the nanofibers to behave like this (Qi et al., 2013). When crab shell chitin was mechanically disintegrated in water by high speed blender, chitin nanofibers with variable widths of 30–110 nm are obtained (Shams *et al.*, 2011). Meanwhile Fan *et al.*, (2008) reported that cationization of the C_2 amino groups present on the crystallite surfaces of the squid pen b-chitin under acidic conditions is one of the most significant conditions for the nanofibers conversion. Shams and Yano, (2013) also reported that in his experiment he found almost 70% of chitin nanofibers have a width within the range of 20–30 nm in acidic condition chitin nanofibers.

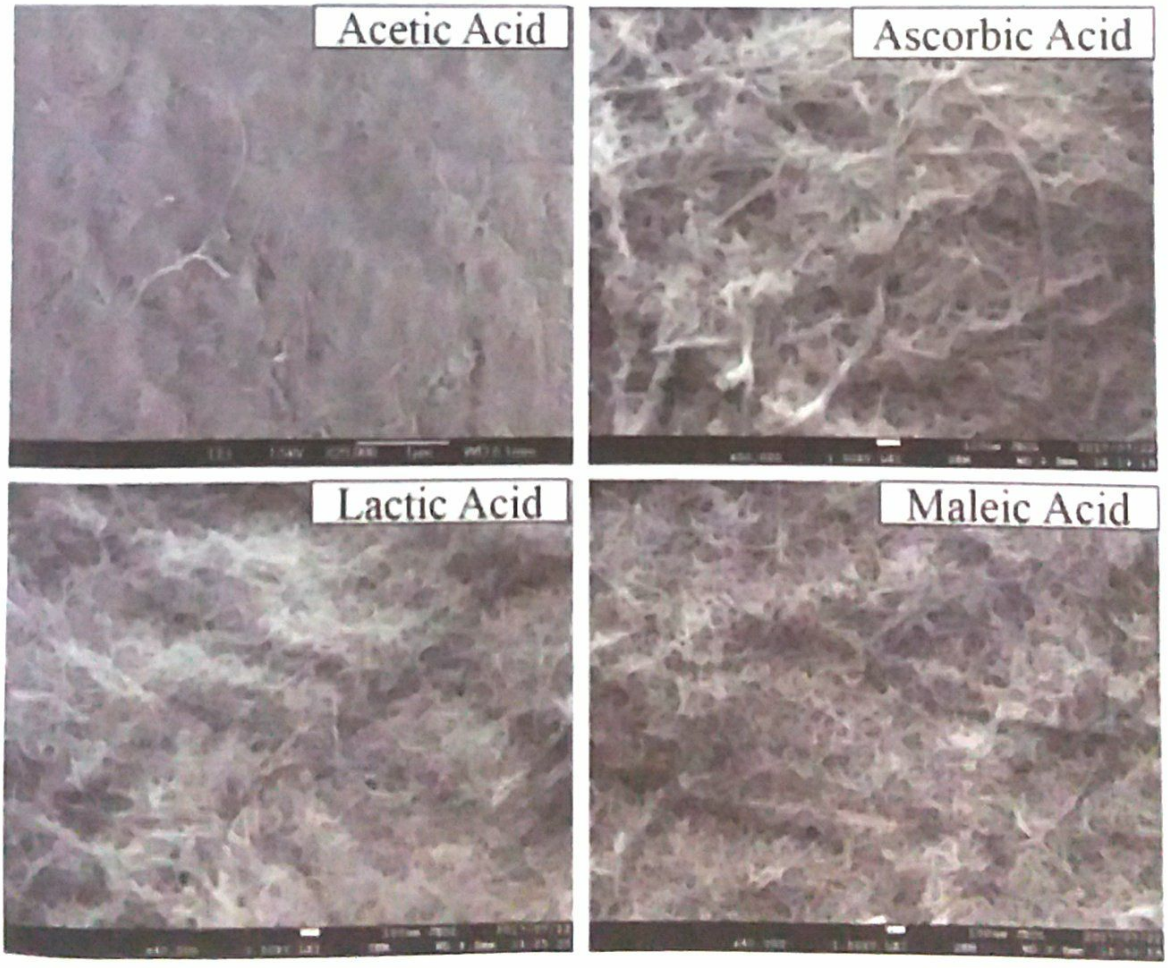


Figure 4.3 FE-SEM image of the nanofibers

The maleic acid and the lactic acid treated nanosheets are aslo represents the same information as the ascorbic acid nanosheet. Qi *et al.,* (2013) found that nanofibrils were observed in the highly transparent PDACh/ ascorbic acid dispersion whereas a considerable amount of bundles were present in the turbid PDACh/ malic acid dispersion. On the other hands the acetic acid condition nanofibers the fibers formed irregular bundles and thick aggregates of fibers. This is due to incomplete fibrillation and the residual pigments during demineralization and deproteinization process of extraction of chitin. The nanofibrils formed irregular bundles of more than 100 nm in width, probably due to the strong H-bonds generated among the fibrils (Biswas *et al.,* 2013). This may be the main cause behind this.

4.1.4 Effect of acid treatments on dewatering time/vacuum filtration

Figure 4.4 shows that the dewatering time is quite more for the ascorbic acidic treated nanofibers. The fibers were dispersed very closely with the water thus makes the dewatering time lengthy. It took 16 minutes on an average for the total dewatering and the lactic and maleic acid treated nanofibers took 15 and 13 minutes respectively. This implies that chitin nanofibers were homogeneously fibrillated under ascorbic acidic condition during mechanical treatment which exposed more surface area by breaking the hydrogen bonds among the nanofibers assisted by electrostatic repulsion force (Biswas *et al.,* 2013). Shams and Yano, (2013) reported that chitin nanofibers took only 20 minutes to be filtered under the acid conditions pH 3-4. But, for the acetic acid condition the dewatering time was less than other acidic condition because of fiber-fiber attraction among the fibers that caused fiber bundles and it took 9 minutes on an average completely dewatering.

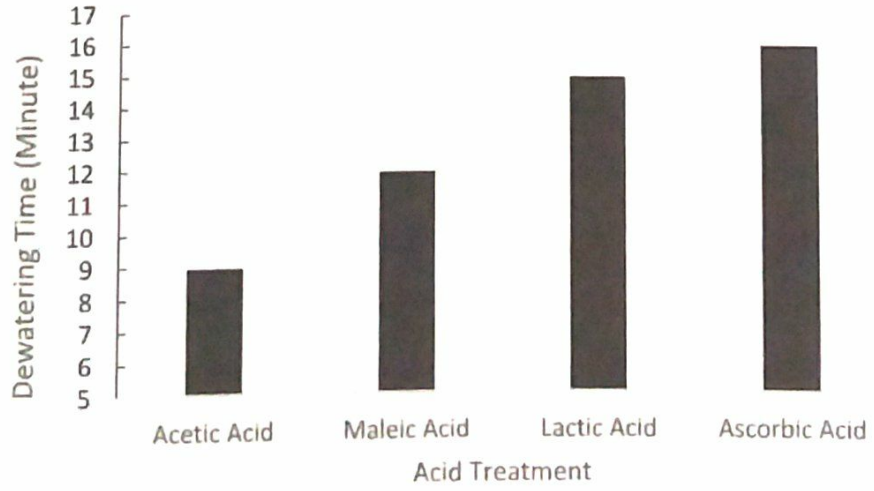


Figure 4.4 Dewatering time of nanofibers during vacuum filtration

4.2 Effect of different acid treatments on partially deacetylated chitin nanocomposites

4.2.1 Effect of acid treatments on light transmittance

Figure 4.5 shows the regular transmittance of four types of acid treated nanocomposites. At a visible wavelength of 600 nm the regular transmittance of the acetic, ascorbic, lactic and maleic acid treated nanocomposites are about to 66%, 51%, 41% and 62% respectively. This is due to the nanofibers width of one tenth with respect to the visible wave length of light. Nakagaito & Yano, (2009) reported that cavities cause light scattering at the nanofiber interfaces, densely packed nanofiber films will have less light scattering, resulting in a high level of transparency. The higher loss in transparency at 400-500 nm range is due to higher filler-width/wavelength ratio (Biswas *et al.*, 2013). Biswas *et al.*, (2013) also reported that in his experiment the regulat transmittance of chitin nano-composite was 84.3%. On the other hand Shams and Yano, (2013) reported a 3 % loss in acrylic resin's regular transmittance at the same range of wavelength using crab CNFs of 20-30 nm in width.

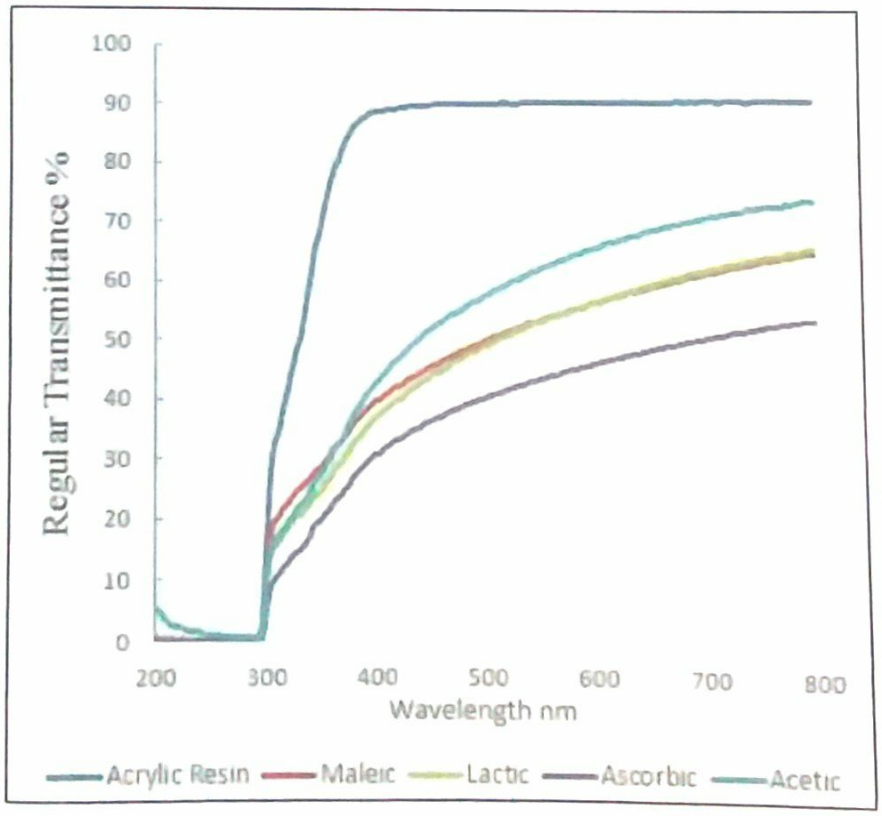


Figure 4.5 Regular light transmittance of nanocomposites

On the other hands figure 4.6 shows the total light transmittance. The total light transparency at visible wavelength of 600 nm the acetic, ascorbic, lactic and maleic acid treated nanocomposites are about to 77%, 81%, 83% and 83% respectively. It was due to an important parameter for transparency of two closely associated materials is the matched refractive index (RI) (Biswas *et al.*, 2013). Qi *et al.*, (2013) found ascorbic acid the highest light transmittance and nanofibrillation yield. Ifuku *et al.*, (2013) reported that the surface-deacetylated NF-chitin/chitosan composite had a higher transparency of approximately 84% at 600 nm than neat surface-deacetylated NF-chitin film regardless of nanofiber content On the other hands the evenness of the nanofibers diameter (20–30 nm) which is much less than the wavelengths of visible spectra (400–800 nm) made it possible to fabricate optically transparent nano-composite with low sensitivity to RI of the plastic matrix (Shams *et al.*, 2011). That is why the total light transparency was much better than regular transmittance.

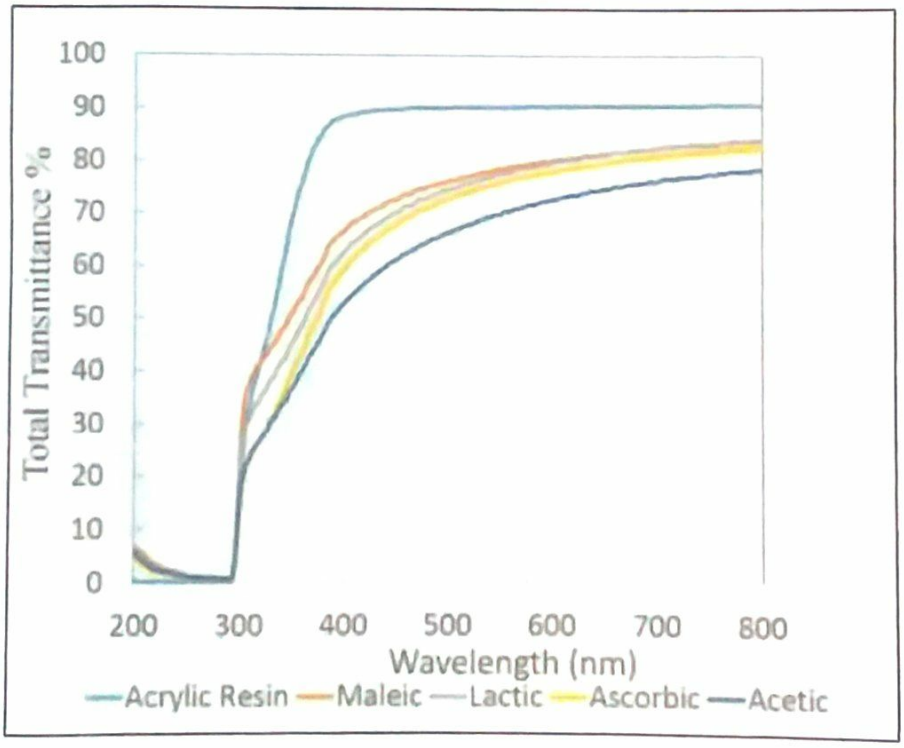


Figure 4.6 Total light transmittance of nanocomposites

4.2.2 Effect of acid treatments on tensile strength

Figure 4.7 shows the MOR of nanocomposites and sheets of four types of acid treatments. In the figure it is seen that the MOR value of the acetic, ascorbic, lactic and maleic, acid treated nanocomposite are 13.7 MPa, 13.3 MPA, 12.5 MPa and 11.2 MPa respectively and the sheets are having the value of 15.4 MPa, 15.5 MPa, 15.8 MPa and 14.1 MPa respectively. On the other hand the MOR value of neat acrylic resin constitutes only 5.5 MPa. This is due to the nanofibrous network in the resin matrix made it stronger and tougher and at the same time increased its bonding. Biswas *et al.*, (2013) reported that in particular, the average tensile strength of chitin/resin nanocomposites increased to 24 MPa from 7 MPa and the fracture strain increased to 0.82% from 0.59%. The high mechanical properties of the film were due to the hydrogen bonding between strong NF-chitin caused by the ambient drying process. In addition, the improvement of mechanical properties would be caused by modification of the deacetylation process (Ifuku *et al.*, 2013)

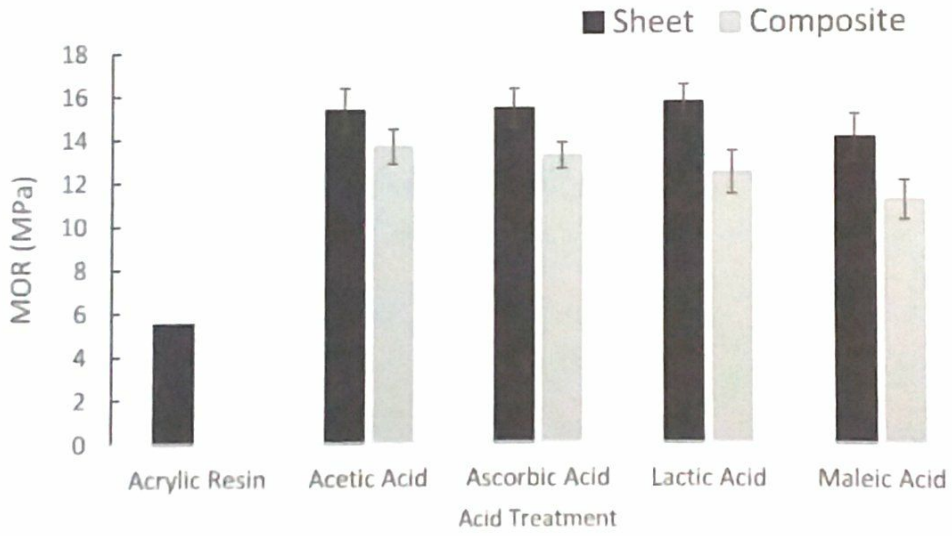


Figure 4.7 MOR of the nanocomposites and sheets

Figure 4.8 shows the MOE value of the nanocomposites and sheets. The figure represents that the MOE value of the acetic, ascorbic, lactic and maleic, acid treated nanocomposite are 1.67GPa, 1.63 GPa, 1.25 GPa and 1.11 GPa respectively the sheets are having the value of 2.3 GPa, 2.6 GPa, 2.02 GPa and 2.4 GPa respectively. On the other hand the MOE value of neat acrylic resin constitutes only 0.2 GPa. This scale increment in strength properties of the nanocomposite is due to the strong network of high strength CNFs. Young's modulus of a CNF with an extended crystalline structure was estimated to be around 150 GPa (Biswas *et al.*, 2014)

Biswas *et al.*, (2013) also reported that the average values of Young's modulus of neat resin films outstandingly increased about 100-fold, i.e., from 0.03 GPa to 3.34 GPa when reinforced with 26 wt% fiber content. The chitin nanofiber-reinforced acrylic resin films exhibited higher tensile strength than neat acrylic resin. In particular, the average values of Young's modulus of the acrylic resin increased from 0.03 GPa to 3.4 GPa (Shams *et al*., 2010)

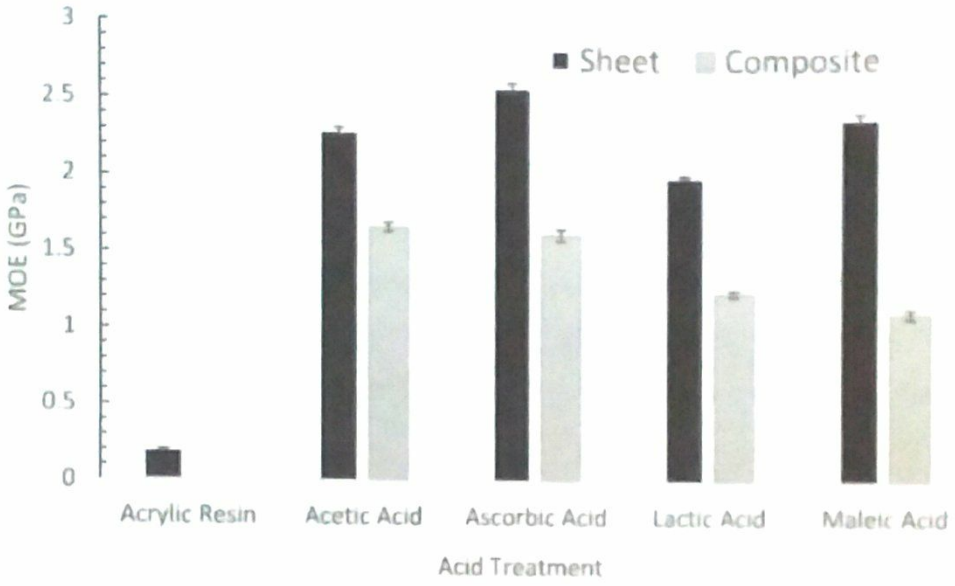


Figure 4.8 MOE of the nanocomposites and sheets

5. Conclusion

Partially deacetylated chitin nanofibers has been successfully extracted in this research from shrimp shells by using simple mechanical treatment under acetic acid, ascorbic acid, lactic acid and maleic acid condition. The extracted nanofibers these four types of acid conditions were different in their characteristics such as viscosity, dewatering time, microscopic image and SEM image. The nano-sheets and nano-composites prepared from nanofibers under these four types of acid conditions showed different tensile properties from one another. The nanosheet under acetic acid and lactic acid condition both showed the MOR value of 15.4 MPa and 15.5 MPa respectively and the MOE value under ascorbic acid condition showed 2.6 GPa. Nano-composites under lactic and maleic acid condition both showed 83% light transmittance whereas the ascorbic acid and acetic acid treated nano-composites showed 81% and 77% light transmittance respectively. All of these perceptions represent strong evidence of the effect of acid treatments on nanofibers. Further study is necessary to improve the properties of nano-composites.

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