Khulna University
Life Science School
Forestry and Wood Technology Discipline

Author(s): Munmun Bandhapadhya

Title: Effects of rotational speed of high speed blender for fabrication of chitin nanofibers from shrimp shells

Supervisor(s): Dr. Md. Iftekhar Shams, Professor, Forestry and Wood Technology Discipline, Khulna University

Programme: Bachelor of Science in Forestry

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Munmun Bandhapadhya
Student ID: FWT-120545

FORESTRY AND WOOD TECHNOLOGY DISCIPLINE
LIFE SCIENCE SCHOOL
KHULNA UNIVERSITY
KHULNA-9208

2017

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THESIS WORK

COURSE NO: FWT- 4114

This thesis paper has been prepared for the partial fulfillment of the requirements of Four (4) years B.Sc. (Hons) degree in Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh

Supervisor,

11/6/17

Dr. Md. Iftekhar Shams

Professor

Forestry and Wood Technology Discipline,

Khulna University,

Khulna, Bangladesh

Submitted By,

Munmun Bandhapadhya

11/6/17

MunmunBandhapadhya

Student ID: 120545

Forestry and Wood Technology Discipline

Khulna University,

Khulna, Bangladesh

DECLARATION

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Munmun Bandhapadhya

DEVOTED

TO MY

BELOVED PARENTS

ACKNOWLEDGEMENT

My first articulate gratitude to almighty God for his blessings upon me for the successful completion of this thesis paper.

I wish to express my heartfelt gratitude and deepest sense of indebtedness to my respective supervisor Dr. Md. Iftekhar Shams, Professor, Forestry and wood Technology Discipline, Khulna University, Khulna for his continuous supervision, guidance, inspiration, valuable advises and thoughtful suggestions during the research period and for providing useful books and papers in preparing and writing up this thesis. Moreover, without his kind supervision and encouragement I could not come up with this paper.

I would like to give my heartfelt thanks to Sourav Bagchi Ratul (MSc in Forestry, Khulna University), Fariha Islam (MSc in Forestry, Khulna University) and Md. Hanif Mia (Laboratory Assistant) for their endless support and cooperation. I am very much grateful to my friend Rehana khatun for help during thesis work at lab.

I am grateful to Jemini Sea Foods Limited, Rupsha, and Khulna, Bangladesh for providing me the shrimp shells. I am also grateful to Research Institute for Sustainable Humnosphere (RISH), Kyoto University, Kyoto,Japan for evaluating the mechanical properties and helps me to prepare my overall result of my thesis work.

Finally, I am very thankful to all of my friends, well-wishers and classmates for their motivation and inspiration during this thesis.

Munmun Bandhapahya

ABSTRACT

Chitin nanofibers were successfully fabricated from shrimp shell waste using simple blending method under different rotational speeds. Matrix from shrimp shell was removed by acid and alkali treatment. The pH was maintained at 3-4 during preparation of nanofibres. From the FE-SEM image and microscopic image it was clear that it is possible to extract chitin nanofibers from 15000 rpm of higher speed blender. The nanofibers obtained from 15000 rpm are small enough to retain the transparency of acrylic resin. By adding nanofibers derived from various rotational speed of high speed blender, the transparency of the composite decreased from 90.5% to 87%. Furthermore, the reinforcement of chitin nanofibers improves the mechanical properties of chitin composites. By adding chitin nanofibers tensile strength increased from 2.5 MPa to 12 MPa and modulus of elasticity increased from 0.3 GPa to 1.3 GPa. Hence, Chitin nanofiber are potential candidate for fabrication of high performance transparent nanocomposites.

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CHAPTER: ONE

INTRODUCTION

Chitin is natural polysaccaride found particularly in the shells of crustaceans such crab and shrimp, the cuticles of insects and the walls of fungi. It is present mainly in crab and shrimp shell, coexisting with protein and some mineral. In 21st century nanotechnology holds enormous promising field in research community for generating and revolutionizing materials, systems and devices with a vast range of applications. Usually, nanotechnology deals with stuff sized 1-100 nm in at least one dimension. With the advancement of nanotechnology, fibrillation of nanofibers come under spotlight for their unique characteristics of minute structure, higher surface area, small range of defects and elevated mechanical properties (Ifuku *et al.*, 2011; Yano, 2005). Among the natural polymers, cellulose nanofibres have gained immense attention as reinforcement in nanocomposites (Abe *et al.*, 2007; Nakagaito and Yano, 2005). The huge amount of shrimp wastes such as the exoskeleton/ shells are the potential source of chitin.

Chitin structure is similar cellulose with an exception is presence of acetoamide (-NH-CO-CH3) group in C2 position instead of hydroxyl group. In addition, chitin is recognized as a bio compatible material with low toxicity, biodegradability, higher crystallinity and hydrophobicity (Shams *et al.*, 2011). High light transmission and fold ability makes chitin promising materials for manufacturing optoelectronic devices like solar cells, bendable displays and so on. Recently, several studies were held for successful fabrication of chitin nanofibres from different source. Chitin is also extracted from a number of other living organisms in the lower plant and animal kingdoms, serving in many functions where reinforcement and strength are required (Rinaudo, 2006). Latest studied have demonstrated that nanofibres can be produced from a number of polymers. Furthermore, as chitin nanofibre consist of an anti-parallel extend crystalline structure, they have excellent mechanical properties, including a high young's modulus, high fracture strength and low thermal expansion (Vencent and wegst 2004; wada and saito 2001).

There is growing interest in producing nanofibres from natural polymers where cellulose is the first abandoned natural source of nanofibres and chitin is second. According to Shams and Yano (2013) optically transparent chitin nanofibres can be fibrillated from crab shell using simplified method. Followed by previous study Subir *et al.*, (2013) reported that around 10^10 to 10^12 tons chitin is bio synthesized annually which are discarded from crab

and shrimp processing industries without effective utilization. After cellulose chitin is considered to be second most abundant natural polysaccharides occurs mainly in the exoskeleton of shellfish. In Bangladesh shrimp production is plentiful and comprises enormous number of shrimp processing industries in the coastal region of the country. These industries process 265000 metric tons shrimp per year (Haq *et al.*, 2009). Most of the waste are discarded and very few are processing low valued fish feed and fertilizer. This huge amount of unprocessed waste causes great extent of environmental pollution in present and will be expand in future. Cellulose is synthesized mainly in plant, where as chitin is synthesized mainly in lower animals, in sea animals, insect and fungi and so on, that increase in size from the simple molecules and highly crystalline fibrils at the nano meter level to composite at the micron level upward (Nogi *et a*l., 2006). It is important to recall that chitin is a natural polymer as well as bio compatible and biodegradable in the body, thus widely used for bio medical and pharmaceutical applications. Additionally, good film forming properties are valuable for wound dressing, artificial skin or packaging. The unique characteristics plus the functionalists of the polymer themselves impart nanofibres with many desirable properties for advanced application (Shams *et al.*, 2011). For example cellulose nanofibres have been shown to be great reinforcement nano composites (Siqueira *et al.*, 2009). Chitin is the naturally abundant and renewable polymers have excellent properties such as biodegradability, bio compatibility, non- toxicity and adsorption. Beside this nanofibres has the unique power of regular light transmittance, better coefficient of thermal expansion, and better tensile strength; this qualities makes the chitin nanofibres more unique than others.

Considering this stunning properties of environmental friendly chitin nanofibres, a utilized the waste produce from the shrimp processing industries an attempt was made to prepare the chitin from shrimp shells. Hence, the purpose of this study was to obtaining chitin nanofibres from shrimp shell wastage using different blending operation.

1.2 OBJECTIVES

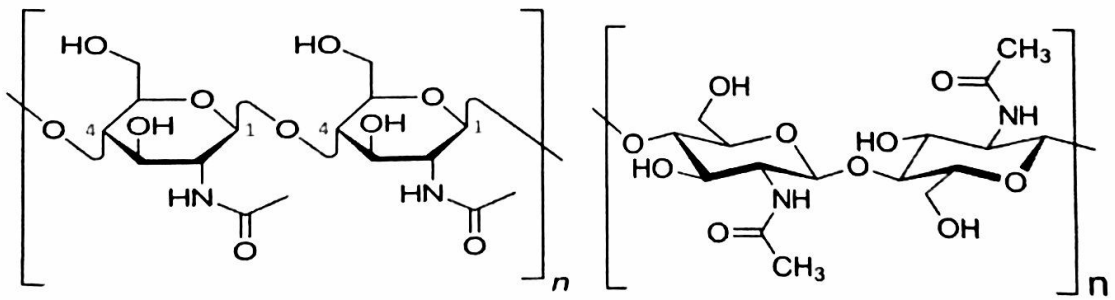
- ✧ To fabricate chitin nanofibres from shrimp shell wastage using high speed blender at different rotational speeds.
- ✧ To prepare chitin nanocomposites from those nanofibers and evaluate their transparency and mechanical properties.

CHAPTER: TWO

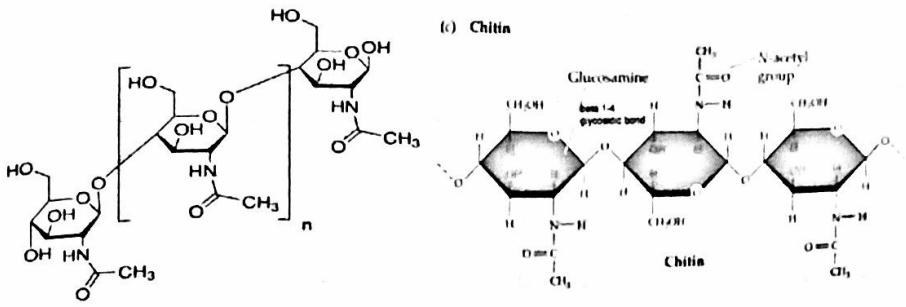
LITERATURE REVIEW

2.1. CHITIN AND ITS STRUCTURE AND PROPERTIES

Chitin is a semi- crystalline bio-polymer which forms microfibrillar arrangement in living organisms. Chitin is a polymer formed primarily of repeating of β (1-4) 2 acetamido 2-deoxy-D-glucose or N-acetyl glucosamine (Nogi *et al.*, 2006). Chitin is nitrogenous polysaccharide, which is white, hard, inelastic, found in the outer skeleton of insect, 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 application ranging from large scale technical application. It is a high molecular weight and linear amino-polysaccharide known to be made of 2-acetamido-2-deoxy-β-D- glucopyranose through a β (1-4) linked structure (Ifuku *et al.*, 2009). The β (1-4)-N- acetyl glycosaminoglycan structure with two hydroxyl groups and an acetamide group makes chitin very crystalline with strong hydrogen bonding (Lertwattanaseri *et al.*, 2009). Chitin is predominantly present as a fibrillar crystalline material.



(a) **(b)**



(c)

FIGURE 2.1 (a) Structure of the chitin molecule, showing two of the *N*-acetylglucosamine units that repeat to form long chains in β-(1→4)-linkage. **(b)** Chitin molecular structure, ©chitin on shrimp shell.

Chitin $(C_8H_{13}O_5N)_n$ is along-chain polymer of an *N*-acetylglucosamine, a derivative of glucose, and is found in many places throughout the natural world. It is a characteristics component of the cell walls of fungi, the mollusks, and the beaks and internal shells of cephalopods, including squid and octopuses and on the scales and other soft tissues of fish and lissamphibians.The structure of chitin is comparable to the polysaccharide cellulose, forming crystalline nanofibrils or whiskers. The chitin nanofibrils consist of nanofibres about 2-5 nm in diameter and about 300 nm length embedded in several protein matrices (Raabe *et al.*, 2012). Since these fibrils are typically embedded in a protein matrix and their diameters range from 2.5 to 2.8 nm, depending on their biological origins [48]. Native chitin can occur in one of three crystalline forms [49]: α- chitin, β- chitin, ϒ-chitin respectively depending on its origin.

2.2 SOURCES OF CHITIN

Chitin was found in animal and plant sources. The major sources of chitin are shrimp, crabs, squilla, lobster, krill, clam, oysters, squid, insects and fungi (Allan *et.al.* (1981).Estimated the chitin content of selected crustacean, insects, moluscan 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 *Aspergillums 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.

Figure 2.2.Sources of chitin

2.2.1. Shrimp production in Bangladesh

Shrimp plays an important role in the economy of Bangladesh. It is the second largest export industries after garments from which Bangladesh earned as US$ 456 million in the year 2006 (Akter *et.al*, 2011). The fisheries sector including shrimp, contributes about 6% to the national GDP and 5% to the national export earnings. Approximately 40,000 metric tons of shrimp are harvested annually from the Bay of Bengal and 18,000 to 20, 000 metric tons of shrimp waste is available annually, whit an extraction but only 30% are extracted (Howlider, 1999). Shrimp waste is a by-product of the shrimp processing industry, composed mainly of heads, tails and shells of shrimp, which are sun-dried or oven dried and ground to a powder. In 1991, the availability of shrimp waste estimated to be 60,000 tons per year in Bangladesh (Haque *et al*., 1993). This shrimp waste is the potential source of chitin.

2.3 CHEMICAL STUCTURE OF CHITIN

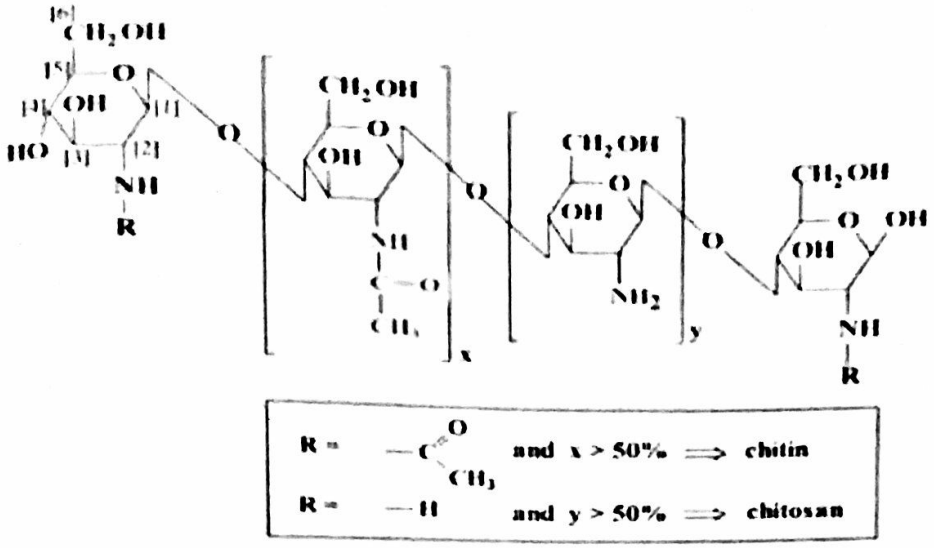


Figure 2.3.1. Structure and properties of chitin

Chitin, poly (β-(1→4)- N-acetyl-D glucosamine) has amino, amide and hydroxyl functional group in its polymer chain. It is the second most abundant naturally occurring polymer right after cellulose. It is synthesized by a mass of living organisms, such as arthropods, fungi, and yeast, with an annual production of 10^10 to 10^11 tons in the world. Chitin has three polymorphic forms, α, β and ϐ, Depending on its source α- chitin is the most abundant form, existing in crab, shrimp, shells, fungal, and yeast walls, and has a highly ordered crystalline structure with an anti-parallel configuration. In con trust, β- chitin is present in squid pen, tube word and diatom spines and has a parallel packing and different crystallographic and parameters from α- chitin. Both of α, & β chitin have strong hydrogen bonding ϐ- chitin is a mixture of α, & β forms. It is important to recall that chitin is a natural polymer as well as biocompatible and biodegradable in the body, thus widely used for biomedical and pharmaceutical applications.

Table 2.3.2.Proposed crystallographic parameters of α & β chitin

Material	a (nm)	b (nm)	c (nm)	γ (°)	Space group
α-chitin	0.474	1.886	1.032	90	$P2_12_12_1$
β-chitin	0.485	0.926	1.038	97.5	$P2_1$

Chitin is a important structural component of many intricately hierarchical structures in natural creatures. An example is the complex structure in arthropods constructed primarily

by chitin. The crustacean exoskeleton mainly consists of chitin, protein and minerals. This bio composite material has a strict hierarchical organization composed of a variety of structural levels. At the molecular level is the chitin itself. Around 20 of chitin molecules are arranged in an anti-parallel configuration to from long crystalline α- chitin nanofibrils with diameters of 2-5 nm.

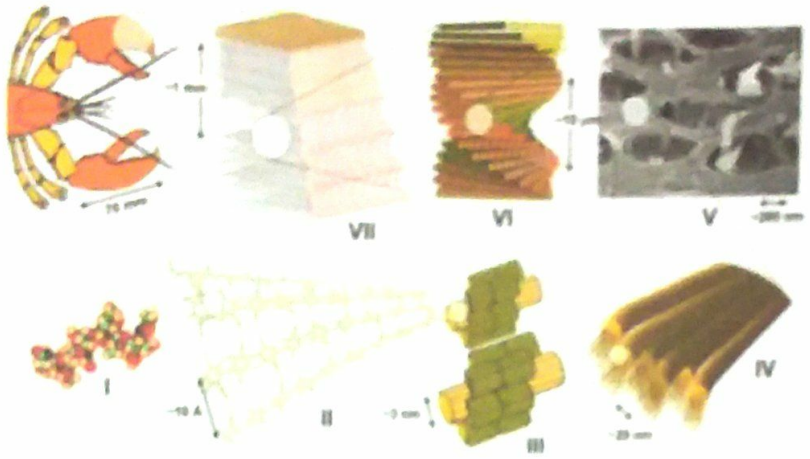


Figure2.3.3. Hierarchical structure Of the exoskeleton material of H.americanus (American lobster) (i) chitin molecule; (ii) anti-parallel packing of α- chitin; (iii) chitin-protein nanofibrils: (iv) chitin protein nanofibers; (v) materialized chitin planes; (vi) twisted plywood structure, and (vii) multilayer cuticle of lobster.

These nanofibrils are wrapped with proteins and are further gathered with other nanofibrils to form large fibers of about 50-300 nm diameter. The next hierarchy level is the formation of a planar woven and branched network created from the chitin proteins and minerals. Purified chitin can be produced from crustaceans and many other and many other organisms by series of chemical treatments, such as acid treatment to remove minerals and base process to deplete proteins.

2.4 NANOFIBRES AND NANOTECHNOLOGY

Nanotechnology as defined by size is naturally very broad, including fields of science as diverse as surface science, organic chemistry, molecular biology, semiconductor physics, micro fabrication, molecular engineering, etc.[4] The associated research and applications are equally diverse, ranging from extensions of conventional device physics to completely new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale to direct control of matter on the atomic scale.

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 molecular scale (Nogi *et al.*, 2006). In its original sense, nanotechnology refers to the project ability to construct items from the bottom up, using technology and tools being developed today to make complete, high performance products. Nanofibres as having a diameter of less than one micron, although the National science Foundation USA, (2005) defines that nanofibres as having at least one dimension of 100 nanometers or less. Nanofibres 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. Now a day's scientists and engineers are finding a wide variety of ways to deliberately make materials at the nanoscale to take advantage of their enhanced properties such as higher strength, lighter weight, increased control of light spectrum, and greater chemical reactivity than their larger-scale counterparts.

CHAPTER: THREE

MATERIAL AND METHODS

3.1 MATERIALS

Shrimp shell was used as main source of chitin nanofibres and obtained from Rupsha sea food industries Ltd. The shrimp shells were combines of body and head shells. Hydrochloric acid (HCl), Sodium hydroxide (NaOH), Ethanol was used for eradicating matrix. A normal blender (Panasonic) and a high speed blender (Vita-Mix blender) were used to fabricate the nanofibres. During this fabrication Acetic acid used to facilitate the fibrillation of nanofibers. Finally, Neat Acrylic resin was used to convert the semitransparent nanocomposites into a transparent one.

3.2 PREPARATION OF CHITIN NANOFIBRES

3.2.1 CHITIN PREPARATION

The chitin of shrimp shells were 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 with tap water and then oven dried at 70 ℃. Then the dried shells were 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.

CHEMICAL DEMINERALIZATION

Demineralization consists in the removal of minerals, primarily calcium carbonate. Demineralization is generally performed by acid treatment using HCl. Demineralization is easily achieved because it involves the decomposition of calcium carbonate into the water-soluble calcium salts with the release of carbon dioxide as shown in the following equation:

$$2\,HCl + CaCO_3 \rightarrow CaCl_2 + H_2O + CO$$

Most of the other minerals present in the shellfish cuticle react similarly and give soluble salts in presence of acid. Then, salts can be easily separated by filtration of the chitin solid phase followed by washing using deionized water.

The sample obtained was demineralized using 35% 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. Then the water was removed gradually from the demineralized sample for further steps.

DEPROTEINIZATION

The deproteinization step is difficulty due to disruption of chemical bonds between chitin and proteins. Then the demineralized shells were ready for the deproteinization process to remove protein from the shell. A solution of 6% NaOH which contains 60g NaOH and 940gm of water was prepared. Then the demineralized shells were mixed with the NaOH solution in a kettle was put into a water bath and boiled at 60 ℃ 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 pigment are removed. The chemically processed demineralized and deproteinized shells were then added with 200g of ethanol in a beaker 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 11%.

PREPARATION OF CHITIN NANOFIBERS

The purified wet sample (chitin) was dispersed in distilled water with a concentration of 1 wt% forming slurry. The slurry was then stirred for 6 hours and several drops of acetic acid were added into the slurry to reduce the pH value between 3-4 ranges. This addition of acetic acid into slurry facilitates the fibrillation and homogeneous dispersion of chitin nanofibres. Afterward, the sample is blending in different blending speed in (Vita -Mix Blender, Osaka chem. Co. Ltd). Such as 1000, 5000, 10000, 15000, 25000 rpm for 10 minutes.

3.2.2 PREPARATION OF CHITIN NANOSHEET

The fibrillated chitin nanofibres were neutralized and dispersed into distilled water at 0.1 wt% of nanofibres contents. From that 1 wt% sample, 5 g sample was taken and mixed with water for making of 0.1 wt% solution to make a nano-sheet. Then this 0.1% was taken in a beaker and kept on magnetic stirrer for about 1 hour to fibrillate the fibers. Then the following steps were done for making the nano-sheets.

DEWATERING AND VACUUM FILTRATION

The suspensions were vacuum filtered using filter papers (pore size: 0.3μm) 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 different time for the different rotational sample.

WET SHEET FORMATION

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

HOT PRESSING

The wet sheet with mesh then instantly taken for hot pressing. During hot pressing 2 MPa pressure and 103±2 ℃ temperature was maintained for 8 minutes for making nano-sheets, after 8 minutes the sheet was taken out and the chitin nanosheets were ready for final analysis.

3.2.2.1 CHARACTERIZATION OF CHITIN NANOFIBERS

VISCOSITY

Viscosity is tested in 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 solution. Viscosity of those chitin nanofibres sample were tested by VTE-03 viscometer using number 4 rotor. Four suspensions of 460g of chitin slurry were prepared based on different blending operation.

MICROSCOPIC IMAGE OBSERVATION

For obtaining microscopic image chitin nanofibers are put on a microscope and observed the fiber orientation of the nanofibers. Several photographs of microscopic image were taken for experiment.

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

The chitin nanosheet 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).

CHAPTER: FOUR

RESULT AND DISCUSSION

4.1 CHARACTERIZATION OF CHITIN NANOFIBRES

Shrimp shell contains strictly hierarchical organization of several substance. The anti parallel alignment of chitin molecules leads the alpha chitin crystals into chitin nanofibres having diameter 2-5 nm (Shams and Yano, 2013).

a b c d e

Figure 4.1. Appearance of chitin slurry (1%) subjected to different rotational speeds of high speed blender.

Figure 4.1 shows the nanofibres in different blending speed. The production of chitin nanofibres from shrimp shells is a systematic process to achieve the target objects. As the shrimp shell consists of 30-40% chitin ,35% protein, 30% CaCO3 and 5-10% lipids (Sajomsang *et al.,* 2009), it was needed to remove that particles except chitin from the shrimp shell to get the targeted chitin. Chitin molecules were aligned in an anti-parallel manner that leads that lead to α- chitin crystals in the form of nanofibres of about 2-5 nm in diameter (Shams *et al.,* 2001). These nanofibres are clustered into long chitin protein fibres with widths of 50-250 nm and are embedded in a variety of proteins and minerals (Raabe *et al.*, 2006).

To extract chitin nanofibres efficiently from such a polymer complex, chitin can be isolated from the cuticle by a series of decalcification and deproteinization steps using acid and alkali treatments, respectively (Shams *et al.*, 2011). The yield of chitin was 11%. After that, the chitin was kept in a wet condition by forming 1 wt% suspension. The pH value to 3-4 was

maintained by adding several drops of acetic acid and stirred for 6 hours to ensure homogeneous dispersion of chitin fibers. Therefore, five types blending operations were done to facilitate the fibrillation. During chemical treatment, some acetoamide groups (-NH-CO-CH3) at C2 position of chitin molecules are converted into deacetylated amino groups (-NH3). In acidic condition, these amino groups (-NH3) converts into positive charges (-NH3+) on nanofibres surface which create an inter-fibrillar electrostatic repulsion force in water (Shams *et at.*, 2011). This force breaks the strong hydrogen bond between the nanofibres and ensures homogenous dispersion of nanofibers.

4.2. Precipitation Rate of suspension

Suspensions of wt% chitin having different blending operation were kept in five test tubes to measure the degree of fibrillation. Precipitation rate of normal blended rotation (1000, 5000, 10000 rpm) slurry was observed very fast when blending rate was raised (rotation 15000 rpm) its takes times to precipitation, when blending speed (rotation 25000 rpm) slurry was remaining unchanged after two weeks. This occurred because of obtaining colloidal structure after 15000 rpm rotating speed as demonstrated by Shams and Yano (2013).

4.3. Viscosity

Viscosity was increased with the rotation speed associate with different rotational operation. The viscosity differs with the morphological characteristics of nanofibres. Thinner and shorter fibers can intertwine more frequently than long fibers. Viscosity varies in different rotational speed. The figure 4.3 represents that the viscosity of 37000rpm is 18 mP.s, 25000 rotational speed (4 number rotor) is 12.9 mP.s, 15000 rotational speed is 7.8 mP.s, 5000 rotational speed 2.5 mP.s, 1000 rotational speed 1.9 mP.s. This is happened because of the concentration, attractive force, and particle size of the solution. The viscosity differs with the morphological characteristics of nanofibers. Thinner and shorter fibers can inter wine more frequently than long fibers.

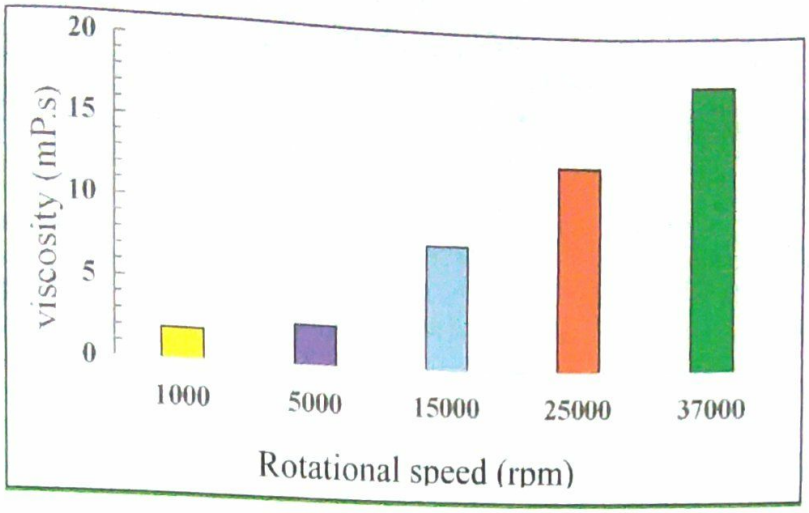


Figure 4.3. Effects of rotational speed on the viscosity of chitin nanofibers

4.4. Microscopic image observation

To understand the fibrillation, firstly microscopic image were observed. From the microscopic view, it was clearly observed the difference within rotational blender The fibers were not fibrillated properly and showed bundle of fiber in different blending speed.

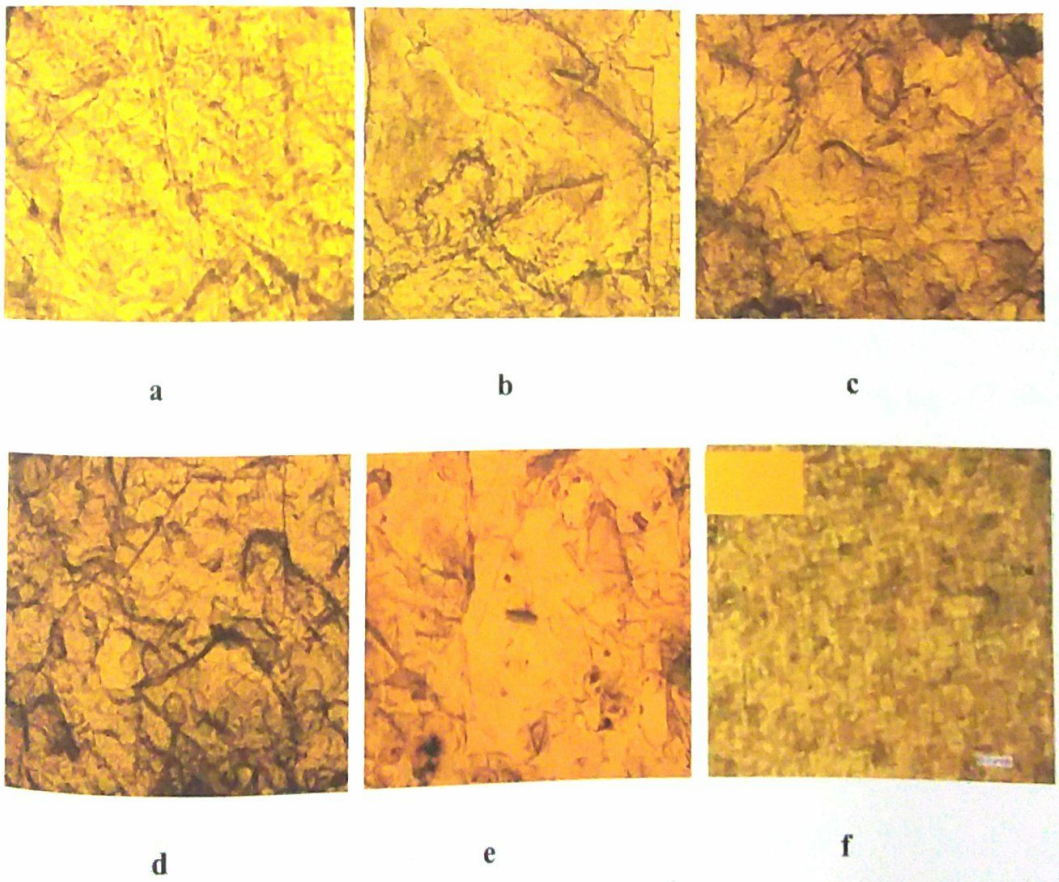


Figure 4.4. Microscopic view (×10) of chitin nano-sheets in different rotational speed of high speed blender. (a.1000, b.5000, c.10000, d.15000, e.25000, f. 35000 rpm)

25000 rotational speeds showed more homogeneous dispersion of fibers and seen small fibers bundle in microscopic view (From figure 4.4). .There is visual difference present among different blending speed. Others shows big bundle of fibers.

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

The figure 4.5 represented that well constracted chitin nanofibers having uniform width. Fibers width of high rotational speed is more uniform (25000, 37000), then low rotational speed (1000, 5000, 10000, 15000). It shows big bundle 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). The figure shows that well-constructed chitin nanofibers were fabricated using a high rotational speed. When crab shell chitin was mechanically disintegrated in water by high rotational speed, chitin nanofibers with variable widths of 30-110 nm are obtained (Shams *et.al.* 2011). There are less fiber accumulation and bundles are seen in high rotational speed. At 1000 rpm, chitin seems to be considerably fibrillated. At 1,000 rpm, chitin seems to be considerably fibrillated. Networks with nano-sized, micro and sub-microfibers were observed. Although the ratio of the amino group was very small (6%), the electro static repulsion force arising from the cationic surface charges was sufficient to break the strong hydrogen bonds between the nanofibers. At 5,000 rpm, the chitin fibers were further fibrillated. However, thicker fibers having width soft 100 nm, were still remained in few numbers. At 15000 rpm, most of the fibers was completely fibrillated and having fine nanofibers network. We could not observe big fibers in a large area. Further, significant change of morphology of the fibers were not observed when the rotating speed was increased from 15,000 to 37,000 rpm.

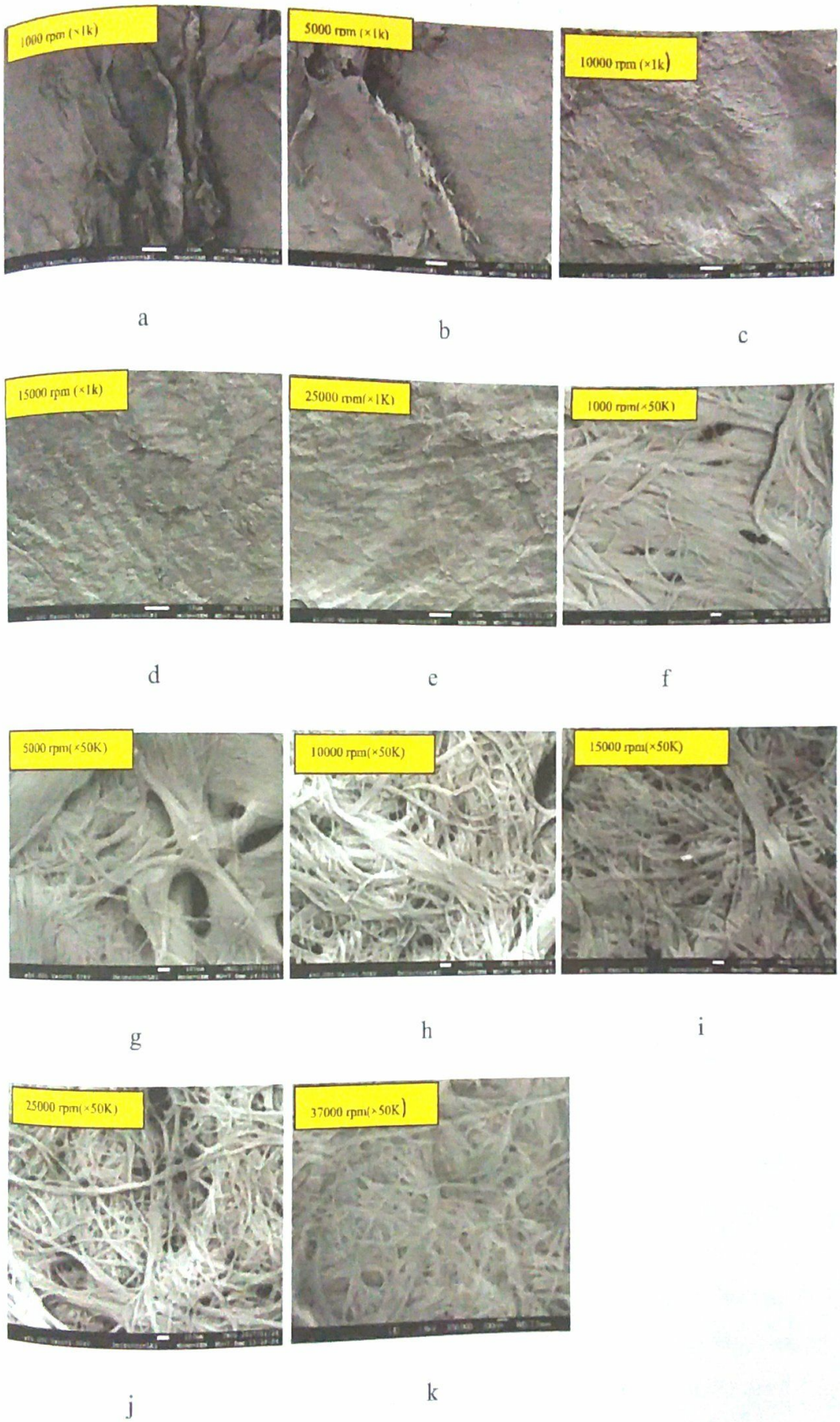


Figure 4.5. FE-SEM image of chitin nanofibers subjected to various rotating speeds of high speed blender

4.6. Dewatering Time

The effect of different blending operations on the suspension was determined indirectly through the dewatering time of suspension. Firstly, fibrillated chitin was dispersed into water at a concentration of 0.1 wt% of water suspension was vacuum filtered during production of chitin nano-sheets. The effect of blending operation on dewatering time took maximum time when rotational speed 25000 and 37000. This might happened due to shortening of nanofibers and increasing of surface area since dewatering time is related to the surface area. Though all the suspension show lower trend of dewatering time in contrast with cellulose nanofibers. The filtration speed of chitin nanofibers is nine times faster than cellulose nanofibers due to the higher hydrophobicity of chitin nanofibers (Biswas *et al.*, 2013).

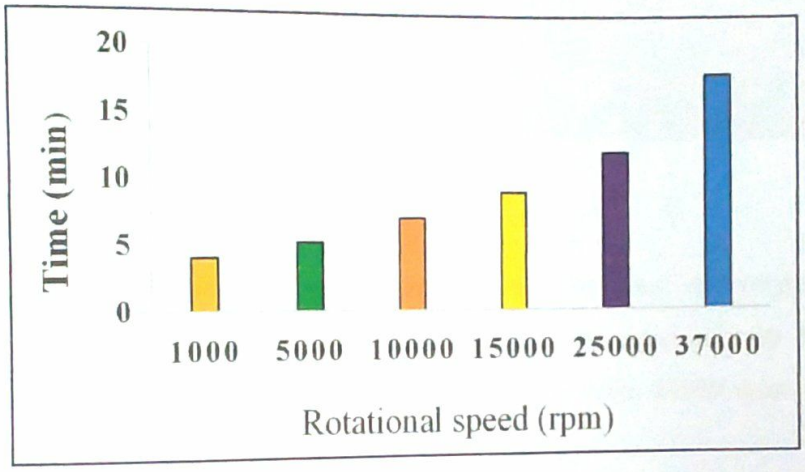


Figure 4.6. Effects of rotating speeds on the dewatering time of chitin slurries to produce sheet of 90 mm in diameter

4.7 Characterization of chitin nano-sheet and optically trnasparent film

The chitin nano-sheets were impregnated into neat acrylic matrix to produce optically transparent chitin nano-composite. When nanofibers are dispersed widely enough throughout a transparent polymer matrix, they can strengthen the polymer, with the resulting composite material retaining its transparency. Due to successfully fibrillation of chitin through high speed blender at (15,000 rpm) the transparency of the composites is reaching above 85%. With increasing rotational speed the transparency of different blending speed also increased. For example at 1000 rpm the transparency 70% on the other hand 15000 rpm the transparency 85%. With increasing rotational speed 15000 to 25000 the transparency increased only 2%. Thus, it seems that at 15000 rpm successful nanofibrillation was

occursas showed in FE-SEM image (4.5). Since chitin slurries are not properly fibrillated at1,000 rpm, big particles concentrated on the sheet and surface of the sheet became rough. On the other hand, with successive fibrillation due to 15,000 rpm of high speed blender, the surface of the sheet was very smooth. Before submerging acrylic resin the appearance of nanosheet is quite different. 1000 rpm showed big particle while 25000 rpm was very smooth. But after submerging acrylic resin the transparency appeared almost uniform result. Because chitin structure of shrimp shell is nano structure.

a b c

Figure 4.7.1. Chitin nano-sheet and optical transparent film after submerging acrylic resin (a.transparency in 25000 rpm blending speed, b. transparency in 1000 rpm blending speed, c. Nano-sheet in before submerging acrylic resin 25000 rpm and 15000 rpm rotational speed

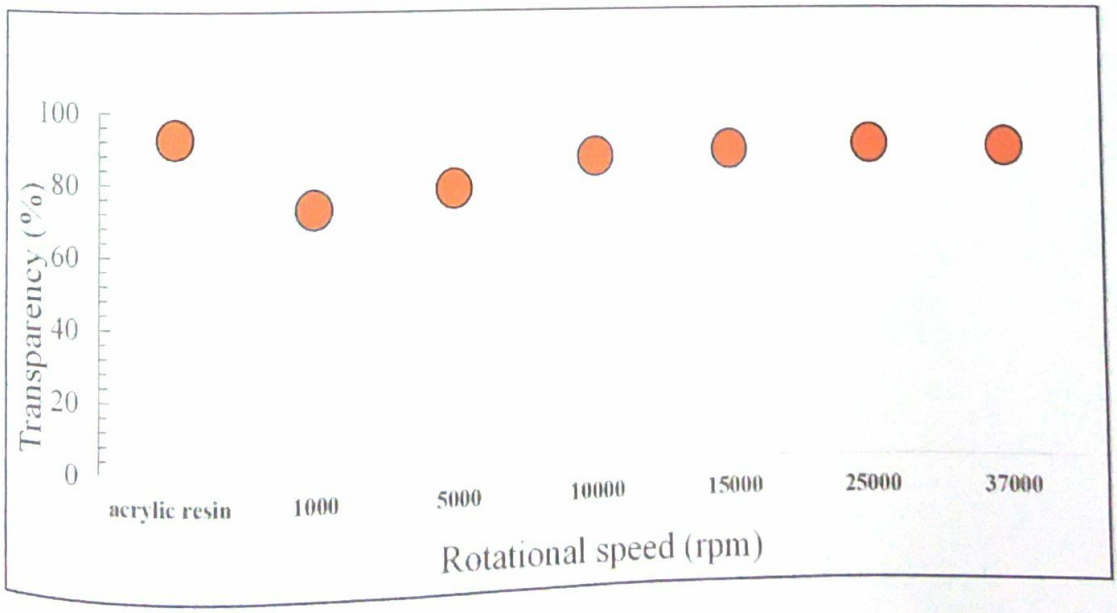
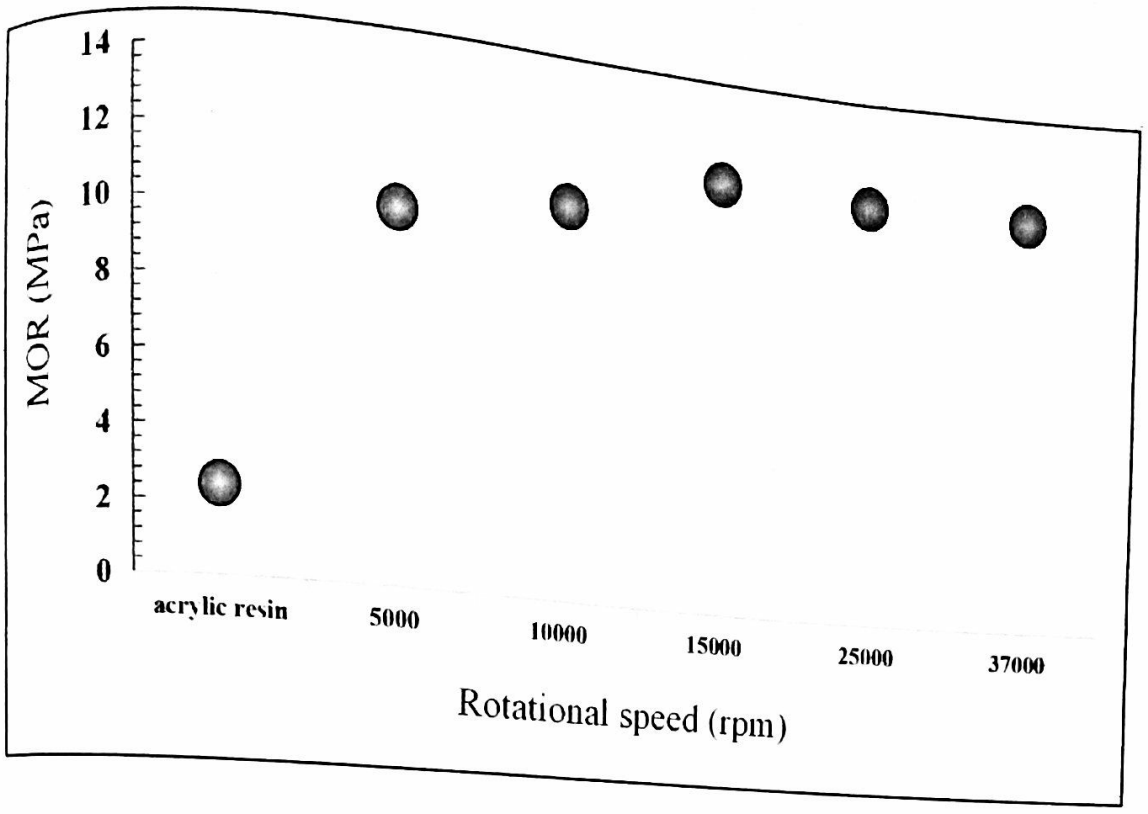


Figure 4.7.2. Effects of Light transmittance various rotational speeds of high speed blender during chitin nanosheet preparation

4.8 Mechanical properties of chitin nanofibers

The modulus of elasticity of pure acrylic resin was 0.2 GPa. By adding nanofiber derived from 1000 rpm of rotational speed, it improved to 1 GPa. Interestingly until 15000 rpm, it became 1.2 GPa and afterward it remains constant (figure 4.8). In the other hand modulus of rupture of pure acrylic resin was 2.5 MPa. After adding nanofiber derived from 1000 rpm of rotational speed, it surprisingly improved to 8 MPa. Until 15000 rpm it became 12 MPa and afterward remains same (figure 4.8). Chitin nanofibers have anantiparallel extended crystal structure, they have a high mechanical strength and Young's modulus. Therefore, chitin nanofibers are considered to be a useful reinforcing element to improve the mechanical properties of composite materials. This may happen due to enhanced homogeneous fibrillation with the enhanced partial rotational speed. Mechanical properties is high when strongly bounded protein is completely removed. Higher protein content is associated with lower degree of nanofiber dispersion, larger agglomerates and lower strain to failure.



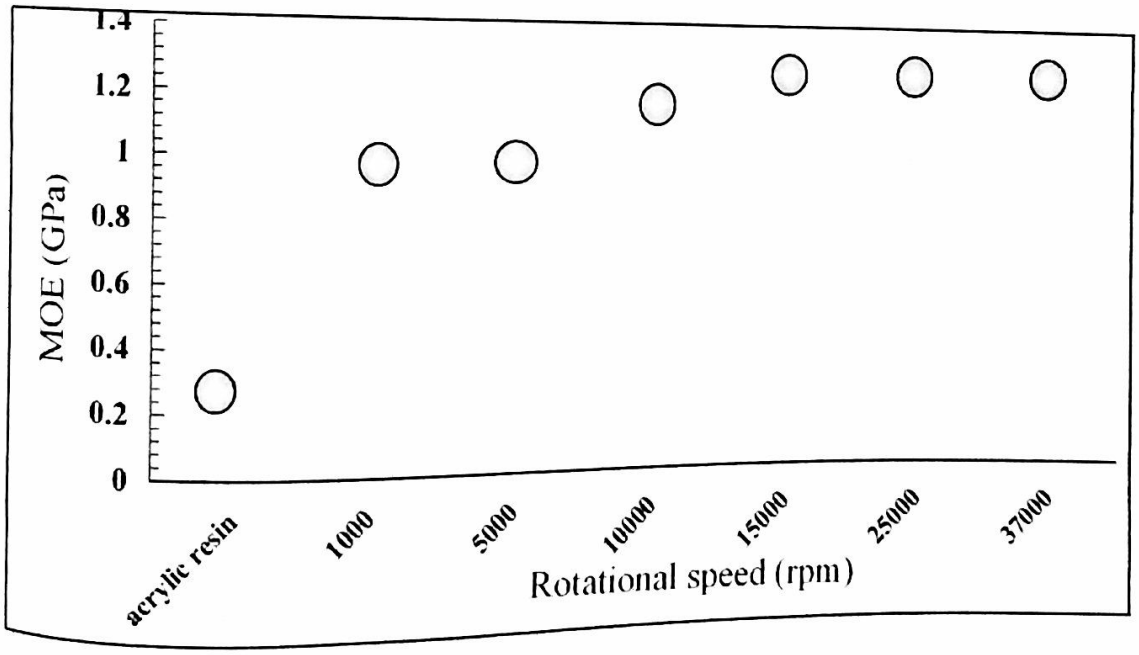


Figure 4.8. Effects of rotational speed of high speed blender on mechanical properties of transparent sheet.

CHAPTER: FIVE

CONCLUSION

Chitin nanofibers have been successfully extracted in this research from shrimp shells by using simple treatment under different rotational speed. From the FE-SEM image and microscopic image it was clear that it is possible to extract chitin nanofibers from 15000 rpm of higher speed blender. This will save the time and energy to produce more uniform nanofibers. The nanofibers obtained from 15000 rpm are small enough to retain the transparency of acrylic resin. By adding nanofibers derived from various rotational speed of high speed blender, the transparency of the composite decreased from 90.5% to 87%. Furthermore, the reinforcement of chitin nanofibers improves the mechanical properties of chitin composites. By adding chitin nanofibers tensile strength increased from 2.5 MPa to 12 MPa and modulus of elasticity increased from 0.3 GPa to 1.3 GPa. Chitin nanofiber are potential candidate for fabrication of high performance transparent nanocomposites.

References

Abe, K.; Iwamoto, S.; and Yano, H. 2007. Obtaining cellulose nanofibers with a uniform width of 15 nm from wood. *Bioimacromolecules*, 8 (10): 3276-3278.

Haq, K. A.; Rahman, S. M. B.; Islam, M. B.; Shaha, B. K.; Ghosh, A. K.; and Sayeed, M. A. B. 2009. Quality aspects of frozen shrimp product in processing industry: A case study in Khulna, Bangladesh. *Bangladesh Research Publications Journal,* 3 (2): 945-962.

Ifuku, S.; Morooka, S.; Nakagaitom A. N.; Morimoto, M.; and Saimoto, H. 2011. Preparation and characterization of optically transparent chitin nanofiber/(meth)acrylic resin composites. *Green Chemistry,* 13: 1708-1711.

Islam, M. S.; Khan, S.; and Tanaka, M. 2004. Waste loading in shrimp and fish processing effluents: potential source of hazards to the coastal and near shore environments. *Marine Pollution Bulletin,* 49: 103-110.

Kose, R.; and Kondo, T. 2011. Favorable 3D-network formation on chitin nanofibers dispersed in water prepared using aqueous counter collision. *SEN'I GAKKASHI,* 67(4): 91-95.

Muzzarelli, R. A. A. 2009. Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. *Carbohydr. Polym.*76: 167.

Nakagaito, A. n.; and Yano, H. 2005. Novel high-strength biocomposites based on microfibrillated cellulose having nano-order-unit web like network structure. *Applied Physics A*, 80: 155-159.

Raabe, D.; Sachs, C.; and Romano, P. 2005. The crustacean exoskeleton as an example of a structurally and mechanically graded biological nanocomposite material. *Acta Mater*, 53: 4281-4292.

Sachindra, N. M; Bhaskar, N.; and Mahendrakar, N. S. 2005. Carotenoids in different body components of Indian shrimps. *Journal of the Science of Food and Agriculture,* 85: 167-172.

Shams, M. I.; and Yano, H. 2013. Simplified fabrication of optically transparent composites reinforced with nanostructured chitin. *J. Polym.Environ*, 21: 937-934.

Shams, M. I.; Ifuku, S.; Nogi, M.; Oku, T.; and Yano, H. 2011. Fabrication of optically transparent chitin nanocomposites. *Applied Physics a,* 102: 325-331.

Subir, K. B.; Atanu, K. D.; Yano, H.; and Shams, M. I. 2013. Development of High Performance Transparent Nanocomposites Reinforced with Nanofibrillated Chitin Extracted from Shrimp Wastes. *J. Chitin Chitosan Sci*, 1: 1–6.

Yano, H.; Sugiyama, J.; Nakagaito, A. N.;Nogi, M.; Matsuura, T.; Hikita, M.; and Handa, K. 2005. Optically transparent composites reinforced with networks of bacterial nanofibers. *Advanced Materials,* 17: 153-155.

Howlider M.A.R. 1999. The Availability of feed and its importance of poultry production. International Poultry Show and Seminar, April 24-26. pp 42-51. 6.

Sajomsang, W., Tantayanon, S., Tangpasuthadol, V., & Daly, W. H. 2009. Quaternization Of N-aryl chitosan derivatives: synthesis, characterization, and antibacterial activity. *Carbohydrate research*, 344(18), 2502-2511.

Haque, A., Nixon, O., and Morris, E. R. 1993. Thermogelation of methylcellulose. Part I: Molecular structure and processes. *Carbohydrate polymer*, 22(3), 161-173.

Akter, M., Rashud, M., Azam, M. G., Howlider, M. a. R., and Hoque, M. A. 2012. Shrimp waste and marine waste as substitute of fish meal in boiler diet. *Bangladesh Journal of Animal Science,* 40(1-2), 18-22.