

**Request for Report for Projects Awarded in 2013 and 2014 by
Mississippi Center for Food Safety and Post-Harvest Technology**

Title: Development of Antimicrobial Packaging Films from Shrimp and Agricultural Post-harvesting Wastes for Seafood Preservation

Award year: 2014

PI: El Barbary Hassan

Co-PI: Barakat Mahmoud

Collaborator:

1. Objectives.

The objective of this proposal was to develop high performance chitosan/nanocellulose films from inexpensive, renewable and natural resources for preserving seafood products, especially catfish. We aimed to prepare applicable bags from these films to measure the actual antimicrobial activity and bacterial growth during preservation of catfish.

2. New Accomplishments toward objectives. Please indicate if all objectives listed were completed.

1. Isolation of cellulose from post-harvesting agricultural wastes and preparation of cellulose nanofibers and whiskers.
2. Characterization of the cellulose nanofibers/whiskers in terms of cellulose morphology. Four different types of cellulose nanofibers including TONc were prepared and characterized.
3. Preparation limited number (5-10 films) from chitosan/nanocellulose packaging films and testing their physical properties, and antimicrobial activity during catfish preservation.

3. Objectives not accomplished and impediments to meeting objectives.

None

4. If continuing project, when will new and/or long term objectives be completed?

April 2016

5. Students supported

- a. PhDs (0.5% FTE, Bawna Soni)
- b. M.S. (None)
- c. Undergraduate (None)

6. Leveraged Funds: External Competitive Funding Applied and Awarded based on findings from this project.
 - a. Applied for:
 - i. Funding agency: Agriculture and Food Research Initiative Competitive Grant Program
 - ii. Program: Improving food quality (Code 1331)
 - iii. Funding request (\$150,000)
 - b. Awarded:
 - i. Funding agency
 - ii. Program
 - iii. Funding awarded (\$\$)

7. Outputs – In addition to the above, please populate the following sections to be included in a report to be compiled in a FSI Research Accomplishment Booklet. The project report will also be posted in a FSI website to be developed.

Project Summary (Issue/Response)

Food packaging is the key process to increase the margin of safety and quality by protecting food during processing, handling and storage. Packaging can also retard food deterioration process, extends the shelf life of processed foods and reduces the risk from pathogens. Recently, seafood has been recognized as high value nutraceuticals, or functional foods. However, seafood products are more perishable than chicken or meat because of their high contents of polyunsaturated fatty acids (PUFAs) and the free amino acids. Therefore, the use of active bio-based films as packaging materials is considered to be one of the most promising ways for effective maintaining seafood quality. The most perspective active bio-film is the one based on chitosan combined with different materials such as plant, animal proteins and polysaccharides. Chitosan is the second most abundant polysaccharide in nature after cellulose and its film exhibits unique physicochemical properties such as biodegradability, biocompatibility, non-toxicity, excellent film-forming ability and high antimicrobial activity against a wide variety of microorganisms, however, chitosan films show unsatisfactory mechanical performance. Incorporating of chitosan with other natural polymers would improve the mechanical properties and other functionalities of the antimicrobial packaging films. Chitosan–nanocellulose blends are of particular interest because of the structural similarity of these two biopolymers resulting in compatible composite materials that combine the physicochemical properties of chitosan with the excellent mechanical properties of cellulose fibers. Therefore, The overall goals of this project is to develop novel seafood packaging films with improved and tailored properties based on chitosan and cellulose nanofibers from agricultural by-products. Our plans to accomplish these goals by: 1) Prepare chitosan from shrimp shell wastes, 2) Prepare and incorporate TEMPO-oxidized cellulose nanofibers with chitosan to produce fully biodegradable high barrier packaging films with antimicrobial activity, and 3) Testing of the prepared films in terms of tensile strength properties, water absorption, water vapor permeability, oxygen permeability and antimicrobial activity.

Project Results/Outcomes

1- Extraction of chitin from shrimp shells and preparation of chitosan

Shrimp shells were obtained from Mississippi State University Coastal Research and Extension Center, which were dried in oven at 400°C for 24 h, grinded and screened into 30 mesh-4mm particle size and the moisture content were determined. Chitin was subjected to several treatment steps such as demineralization, deproteinization, and deacetylation for preparation of chitosan. More than 500g of chitosan were successfully prepared and characterized. The degree of deacetylation for chitosan was determined according to the standard methods and found to be 72%. FTIR and thermogravimetric analysis were performed on oven dried chitosan samples and the results were compared with the authentic commercial chitosan data.

2- Isolation of cellulose and preparation of nanocellulose from Cotton Stalks

Post-harvesting cellulose material (cotton stalks) was obtained from Myron May Farm, Stewart, Mississippi. The air dried cotton stalks were grinded and sieved in hammer mill between 30-80 mesh size sieves which was stored in Ziploc bags for analyses. The compositional analyses for cotton stalks were determined according to NREL/TP-510-42681 standard method. Cotton stalks was subjected to different pretreatment steps (alkaline acid pretreatment and oxidation pretreatment) to remove lignin and hemicellulose and get fully bleached pulp. Cotton stalk bleached pulp was subjected to three different pretreatment steps (ultrasonication pretreatment, acid hydrolysis pretreatment and TEMPO mediated oxidation pretreatment) to produce cellulose nanofibers. The four different produced cellulose nanofibers were fully characterized in regards to surface morphology (AFM and FE-SEM), functional group analysis (FTIR) and thermal gravimetric analysis.

3- Preparation of different chitosan/nanocellulose fiber films.

Based on the characterization results of different cellulose nanofiber, TEMPO oxidized cellulose nanofibers (TOCNf) were chose as the best nanofibers that can be blended with chitosan for the preparation of antimicrobial packaging films. The following preparation parameters have been chosen for the preparation of chitosan/TOCNf films:

Chitosan (wt %)	TOCNFs (wt%)	Sorbitol (wt%)	1% acetic acid solution (mL)	Replicates
100	0	25	100	3
95	5	25	100	3
90	10	25	100	3
85	15	25	100	3
80	20	25	100	3

The moisture content, thermal gravimetric analysis and FTIR were studied for the prepared films but the strength properties, gas barrier properties and antimicrobial activities are still under study. Below are summary for some of our results obtained during our first year study of this project.

Figure 1. FE-SEM micrographs of different extracted CNFs

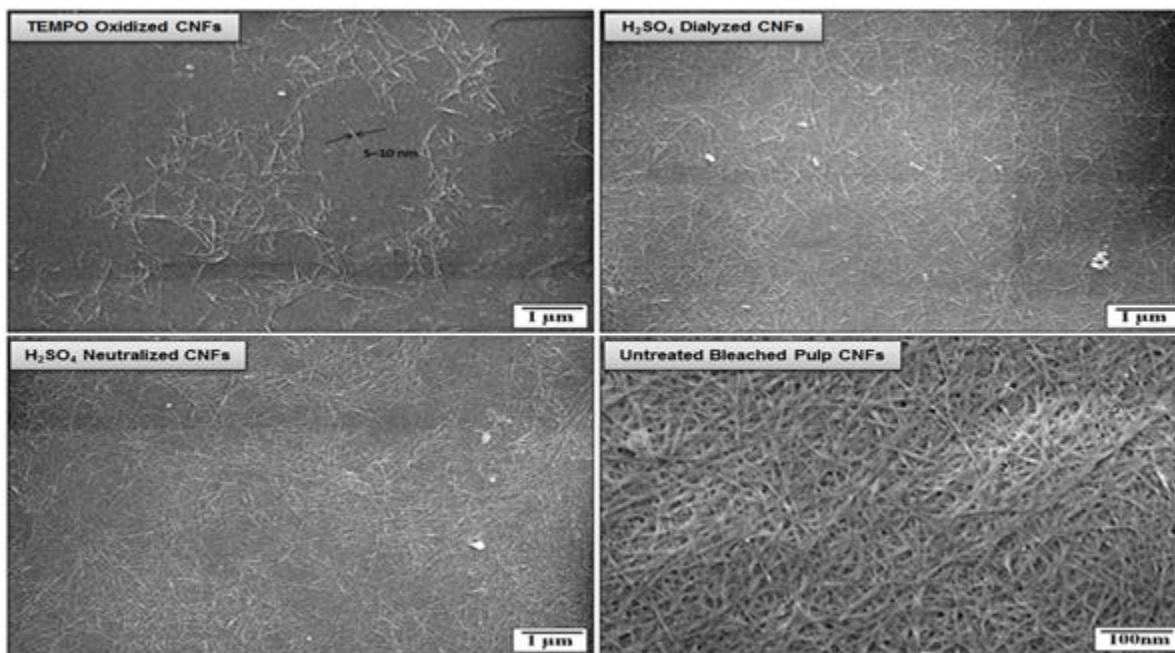


Figure 2. AFM images of different CNFs

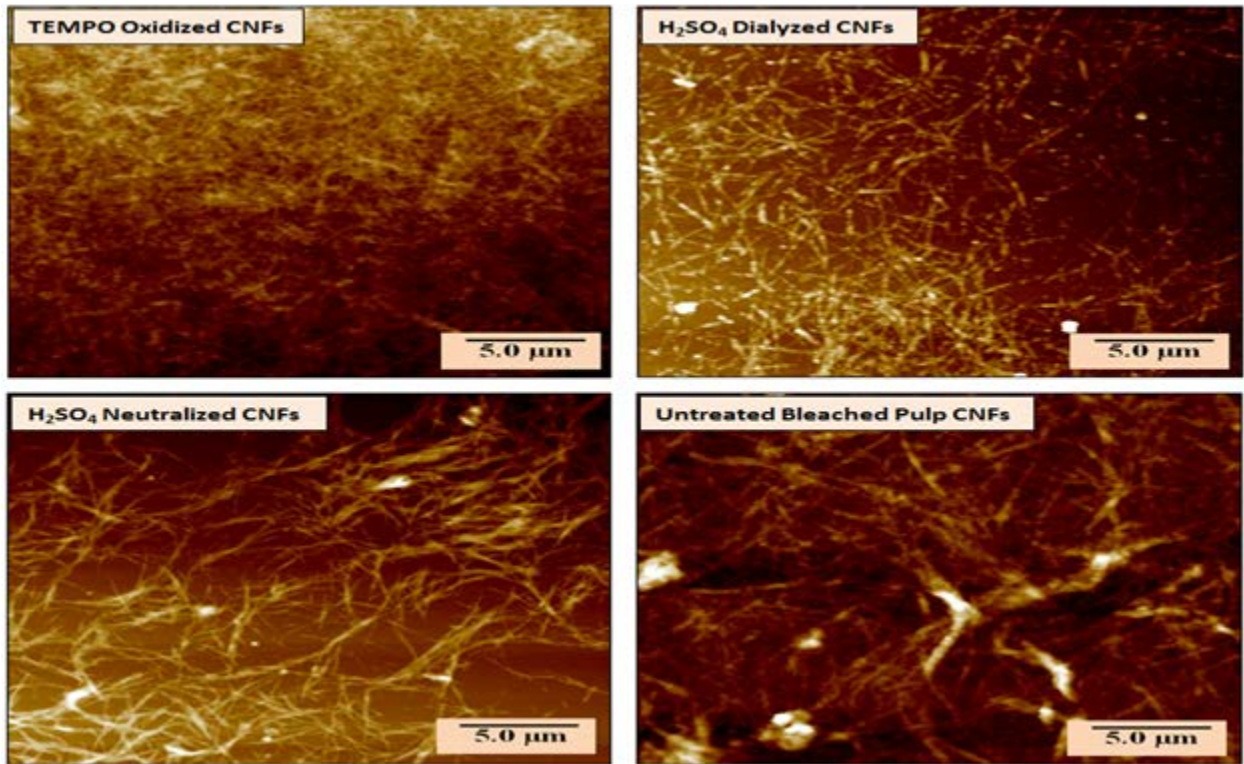


Figure 3. ATR spectra of various chemically treated and untreated cellulose nanofibers

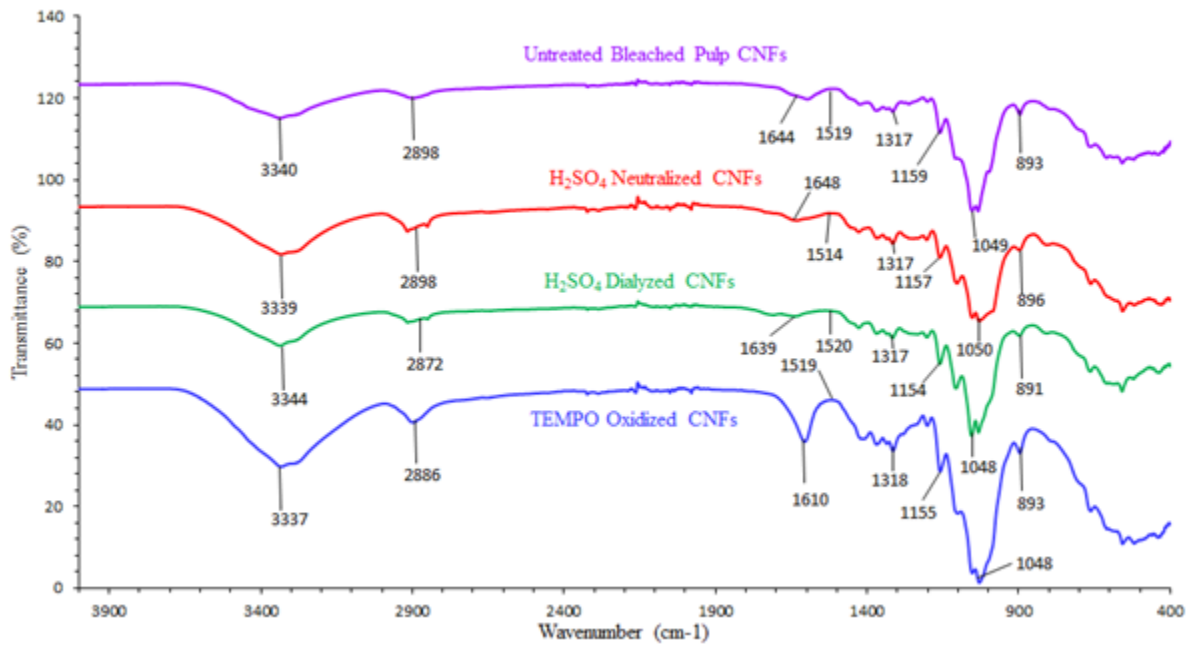


Figure 4. TGA and DTG thermograph of different cellulose nanofibers after each stage of the treatment

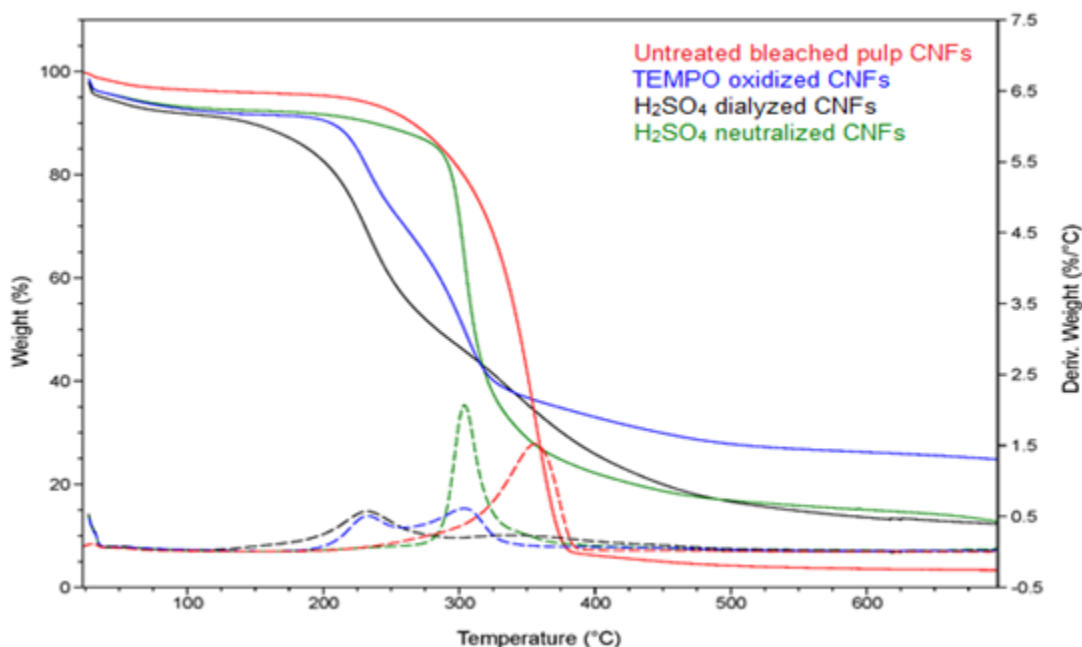


Table 1: Length and diameter difference between four kinds of CNFs

Cellulose nanofibers	Diameter (nm)	Length (nm)	Yield (%)
TEMPO oxidized CNFs	3–20	50–100	90–95
H ₂ SO ₄ Dialyzed CNFs	10–50	100–500	45–50
H ₂ SO ₄ Neutralized CNFs	10–50	200–500	40–45
Untreated Bleached Pulp CNFs	50–500	>1500	>95

Anticipated outcomes

This project will provide a set of data for integrated chemical and processing techniques for preparation of novel antimicrobial biodegradable films for seafood packaging from totally post-harvesting agricultural and shrimp wastes available in Mississippi. Another expected outcome from this project will be the significant reduction in the amounts hazardous wastes associated with using synthetic polymers,

Project Impacts/Benefits

The development of new polymeric materials based on chitosan and cellulose nanofibers represents a particularly interesting and promising strategy with the context of new sustainable and environmental-friendly products derived from renewable resources. The results of this research project will have a great scientific and economic impact on the state of Mississippi and whole U.S. as follows:

1. Asset Mississippi by-products and waste producers to convert their wastes into useful co-products in a manner that eliminate the cost of landfill disposal.
2. Accelerate the development and demonstration of technologies that provide diversity and stability through increasing the dependence on the domestic and renewable resources.

Project Deliverables

1. Soni, B., Mahmoud, B.S. Hassan, E.M. 2014. Preparation and Characterization of Various Cellulose Nanofibers for Improvement of Antimicrobial Packaging Films. 13th Annual Southern BioProducts and Renewable Energy Conference. Hattiesburg, MS. November 13th, 2014.

Graphics

From wastes to useful products

