Study on antibacterial papermaking for food packaging using rice straw nanocellulose and nanochitosan

Thai Dinh Cuong, Nguyen Viet Linh, Nguyen Hoang Chung, Le Quang Dien*, Hanoi University of Science and Technology, 1 Dai Co Viet, Hai Ba Trung district, Hanoi, Vietnam
dien.lequang@hust.edu.vn

Abstract. In this study, the antibacterial bleached hardwood kraft pulp-based paper sheets with a base weight of around 125 g/m² were made with surface sizing by a mixture of oxidated starch and additives from acetic acid-treated nanochitosan and nanocellulose prepared from limited hydrolysis of rice straw by dilute sulfuric acid with added hydrogen peroxide. The characteristics of nanomaterials were analyzed by SEM and XRD. The barrier and antibacterial properties of paper were investigated to assess their ability to contain liquid and food products. Using the sizing mixture which has a solids content of 8% with additives improved the mechanical strength of the paper. The best value of tearing strength of 18.94 mN.m²/g was obtained with adding of 0.5% of nanocellulose and 1.5% of nanochitosan. The burst index of paper reached its highest value of 5.07 kP.m²/g when both nanocellulose and nanochitosan were used at the dosages of 1.0%. The antibacterial features on E. coli clearly showed in papers with 2% of nanochitosan or with the mixture of 1% nanocellulose and 1% nanochitosan addition.

1. Introduction
In the context of the increasing application of renewable sources, biopolymers such as cellulose, starch, and chitosan have been identified as promising feedstocks due to their abundant availability and potential as well as their worldwide distribution [1,2]. Among them, cellulose is the most potential renewable compound as it is easy to extract and process. Cellulose, which can be produced from a variety of raw materials, is a high-quality, low-cost raw material bioproducts production. Cellulose is an attractive polymer with the potentials to be modified and functionalized with many already available industrial uses and there are still many other aspects to discover [3–5].

On the other hand, chitosan is a biopolymer synthesized from chitin, which is the second-largest reserve potential after cellulose. Chitosan is the main component of cell walls in fungi, arthropods, crustaceans, such as crabs, lobsters, and shrimp, insects, mollusks, and fish or amphibians scales [6,7].

Due to the above characteristics, both polymers are receiving great attention for the production of environmentally friendly products and nanomaterials. The use of nanofibrillated cellulose (NFC) for the production of nanocomposites containing inorganic nanoparticles has attracted the attention of the scientific community. NFC fibers have remarkable properties such as high aspect ratio, tensile strength, and modulus that turn this material into a good candidate for reinforcement elements in paper and nanocomposites [8,9]. Also, the application of chitosan in the production of biodegradable packaging is a potential direction in great demand [9,10].
In the field of production and consumption of industrial packaging, using non-biodegradable plastic packages or difficult biodegradable plastic-coated paper today is a challenge. It is a great concern to society and the world because of the obvious risk of polluting land, water, and air, causing serious pollution of habitats and aquatic and ocean systems [11].

These problems promote research on papermaking using biodegradable and antibacterial additives, to replace PE/PP plastic packages and be safe to use as pharmaceutical and food packaging. In this respect, cellulose and chitosan can fulfill the necessary features. Besides that, today, inorganic antimicrobial agents are promising such as metal salts, nano-sized metals, and metal oxides, such as CuO, TiO2, ZnO, Al2O3, SiO2, Fe2O3, and CeO2, which are frequently used as antibacterial agents [12]. Among them, ZnO has excellent antibacterial activities, and its application field is very broad which is applied for antimicrobial paper making by surface sizing.

The aim of this study was to produce antimicrobial packaging papers using rice straw nanocellulose and nanochitosan as additives for internal sizing.

2. Materials and methods

2.1. Materials

Oryza sativa L. rice straw (RS) harvested in the North of Vietnam was used in the experiments. The air-dried rice straw was ground and screened. The fraction retained on a 24-mesh screen was used for the study. The prepared material was then stored in plastic bags at room temperature and did not need to be washed before use.

Commercial bleached kraft pulp with a brightness of 84.6% ISO was produced and supplied by An Hoa Paper JSC (Tuyen Quang province, Viet Nam). Commercial chitosan was purchased from Chitosan VN JSC (Kien Giang province, Viet Nam).

Chemicals, including sodium hydroxide, hydrogen peroxide, and sulfuric acid, were all analytical grades and originated from Sigma Aldrich.

2.2. Nanocellulose preparation from rice straw

To obtain cellulose, rice straw was treated with a sodium hydroxide solution at a dosage of 12 wt% over the oven-dried mass of rice straw. The reaction took place in a 5-L autoclave for 180 min at 120°C. The temperature was raised from room temperature (about 30°C) to 120°C within 35 min. The obtained cellulose pulp was then washed, dewatered, and bleached in two stages with hydrogen peroxide at a dosage of 6 wt% and at 70°C to achieve a brightness of 81.6 % ISO.

For nanocellulose fabrication, bleached RS cellulose was first hydrolyzed with an aqueous mixture of 5wt% H2O2 and 5wt% H2SO4 for 180 min at 150°C, liquor to solid ratio of 10:1 (mL/g). After that, the nanocellulose was cleaned and treated with a dilute solution of hydrogen peroxide and sodium hydroxide. The mixture was then washed with distilled water before being gently refined for 2 min with a laboratory OSAKA® multifunctional cutter model DH-807 (350W, fixed rotation of 2500 rpm). The treatment was carried out to gain a homogeneous suspension of nanocellulose that did not precipitate after a long period of storage (i.e., more than 48 h) [14]. Obtained nanocellulose was dewatered and used as a paper additive. The procedure for nanocellulose preparation is illustrated in Figure 1.

For analysis of morphology structure, nanocellulose was dissolved in 96% ethanol and characterized using a Scanning Electron Microscope (JEOL JSM 5410 LV FESEM).

2.3. Preparation and characterization of nanochitosan

Impurities in commercial chitosan such as proteins and salts were eliminated according to the following procedure: 5 g of chitosan was dissolved in 500 mL of 2 wt% aqueous acetic acid for 24 h with stirring. The resulting nanochitosan solution was passed through Whatman cellulose nitrate filters, respectively using a vacuum filtration system. To precipitate the nanochitosan, a 10 wt% sodium hydroxide solution was added dropwise to the purified chitosan solution until neutralization (pH ~ 7) was achieved. The nanochitosan was then washed 6 times with distilled water and three times with 96% ethanol to eliminate any remaining salts. The resulting solid material was used for papermaking as an additive.
For analysis of morphology structure, chitosan was dissolved in 96% ethanol and characterized using a Scanning Electron Microscope (JEOL JSM 5410 LV FESEM).

2.4. Papermaking and evaluation of mechanical and barrier properties of paper sheets

Paper sheets with a basis weight of around 125 g/m² were first made from bleached kraft pulp on a laboratory papermaking machine. Previously synthesized nanocellulose, nanochitosan or a combination of the two in various ratios were used as paper additives during the paper making procedure.

Each paper sheet was determined its basis weight. Paper properties were analyzed by standard methods as follows: tensile strength (TAPPI T404 wd-03), burst strength (TAPPI 403 om-10).

![Figure 1. Production of nanocellulose from rice straw; (1) Rice straw, (2) Cellulose pulping and unbleached cellulose pulp, (3) Bleached cellulose, (4) Cellulose hydrolysis, and purification, (5) Nanocellulose refining and sample preparation](image)

2.5. Assessment of antibacterial activity of paper

The assessment of antibacterial activities of paper samples was conducted by using the method described by Hadacek and Greger [14]. *E. coli* was cultivated within 20 h in a liquid medium with regular shaking at 37°C. At the end of the incubation period, the bacteria were extracted from the samples under investigation by using a neutralizing solution. The number of living cells (CFU=colony forming units) in the extracted suspension was evaluated by the count plate agar method.

3. Results and discussion

3.1. Nanocellulose and chitosan characterization

The rice straw containing 78.64% of cellulose, 4.25% of lignin, 11.37% of pentosan, and 0.27% of ash was then subjected to the nanocellulose preparation. Under the selected conditions of cellulose and nanocellulose preparation process from rice straw, the yield of bleached cellulose and nanocellulose
were 38.62% and 77.81%, respectively. According to SEM analysis (Figure 2A), the nanocellulose fibers have an average diameter of less than 100 nm.

Nanochitosan was fibrillated and remained in an aqueous solution of acetic acid. The fibrillation and longest solution remaining status of chitosan in an aqueous acetic acid solution was obtained at room temperature, at a low solvent-to-nanochitosan ratio. The smallest particle size of about 50 nm is obtained (Figure 2B). With small dimensions, nanochitosan is floated and distributed on the surface of the solution. Indeed, at small particle sizes, nanochitosan agglomerates and remains on the surface of the liquid once added to the acid solution. As a result, there is no complete dispersion of the solids in the liquid phase and solubilization was slow. These observations were independent of the stirring power used to mix the solid and liquid phases.

Figure 2. Scanning electron micrographs at a 100,000x magnification of rice straw nanocellulose (A) and nanochitosan (B)

Analysis of XRD of nanomaterials (Figure 3) showed the nanocellulose powder exhibits a broad characteristic peak at 20° (2θ). For nanochitosan, characteristics peak at 20° and 27°, which is indicative of the predominantly amorphous form of chitosan [13]. These XRD characteristics of nanocellulose and nanochitosan were not different from rice straw cellulose and native chitosan. It showed good nanofibrillation and was not no reaction occurred with the functional groups of cellulose and chitosan. The peak intensity of nanocellulose was higher than that of cellulose. It showed the crystallinity of nanocellulose higher than that of cellulose. The peak intensity of nanochitosan was equivalent to that of chitosan.

Figure 3. X-ray diffractogram of rice straw nanocellulose (A) and nanochitosan (B)
3.2. Application of nanocellulose and nanochitosan as paper additives

The influences of nanocellulose and nanochitosan on paper mechanical properties are presented in Figure 3. As seen from the obtained results, increasing the dosages of nanocellulose from 2 to 10% reduces the breaking length and tensile index of the papers by approx. 8.4% and 19.6%, respectively. The main reason is that the rice straw nanocellulose fibers are much shorter than bleached kraft pulp and most of the hemicellulose was dissolved during the production of nanocellulose. Shorter fibers with fewer functional groups that can create hydrogen bonds during the formation of paper result in the reduction of the tensile index of the paper. Furthermore, the presence of nanocellulose improves paper density, therefore, the burst index raises with increasing nanocellulose addition. However, at 7% and above of nanocellulose addition, the burst index significantly drops to be lower than that of the sample without nanocellulose added. This suggests that when the percentage of nanocellulose in pulp suspension was higher than 7%, the presence of nanocellulose negatively affects the bonding among fibers.

The influences of nanochitosan on paper properties are slightly different (Figure 4). Compared to nanocellulose, nanochitosan strongly improves the breaking length of the paper. Maybe, this is because the mechanical strength of nanochitosan is greater than nanocellulose, although nanochitosan has a shorter fiber length than nanocellulose. Tear index also follows the same trend as breaking length does. At the nanochitosan dosage of 2%, the burst index is significantly improved and reached the highest value of 4.85 kPa.m²/g. This comes from the fact that nanochitosan fibers are more uniform than nanocellulose extracted from rice straw, leading to the higher paper density, and burst index.

In summary, when being used separately, nanochitosan shows more advantages than nanocellulose. Nanochitosan provides papers with better mechanical and barrier properties at the dosage of 2% over the dried mass of pulp.
On the other hand, adding both RS nanocellulose and nanochitosan to the pulp can significantly improve these three mechanical properties of paper (Table 1). By adjusting the ratio between RS nanocellulose and nanochitosan, it is possible to obtain paper sheets with desirable quality. The trend, in this case, is different than when the two materials were used separately. For example, at the dosages of 4% of nanocellulose and 1% of nanochitosan over the dried mass of pulp, the breaking length of the paper sample is significantly improved and reaches the highest value of 6146.9 m. However, the best value of tearing strength is 18.94 mN.m$^2$/g when using 0.5% of nanocellulose and 1.5% of nanochitosan. Burst index reaches its highest value of 5.07 kPa.m$^2$/g when both nanocellulose and nanochitosan were used at the dosages of 1.0%.

### 3.3. Assessment on antibacterial properties of paper

Four samples were selected for the test including paper added with 2% of nanocellulose, with 2% of nanochitosan, with a mixture of 1% of nanocellulose and 1% of nanochitosan, and a paper without addition as the control sample. The results are presented in Figure 5. As can be seen, papers with 2% of nanochitosan (Figure 5C) and with a mixture of 1% of nanocellulose and 1% of nanochitosan (Figure 5D) are antibacterial with *E. coli*. Obviously, the control sample (Figure 5A) shows no possibility of an antibacterial feature. The paper with 2% of nanocellulose addition shows a weak antibacterial property to *E. coli*. Solid content of the white water demonstrates that the retention rate of nanocellulose on paper is worse than that of nanochitosan. It, therefore, influences the mechanical properties of paper. This also suggests that it is necessary to use these materials for coating on paper to improve their binding capacity on paper’s surface, leading to the improvement in surface strength and enhancement of antibacterial characteristics of the paper.

| No | Rice straw nanocellulose (%) | Nanochitosan (%) | Basis weight (g/m$^2$) | Breaking length (m) | Tear index (mN.m$^2$/g) | Burst index (kPa.m$^2$/g) |
|----|-------------------------------|------------------|------------------------|---------------------|-------------------------|--------------------------|
| 1  | Control                       | Control          | 125.5                  | 5995.3              | 12.42                   | 3.64                     |
| 2  | 0.5                           | 1.5              | 125.4                  | 5300.5              | 18.94                   | 3.69                     |
| 3  | 1.0                           | 1.0              | 125.4                  | 5495.0              | 17.16                   | 5.08                     |
| 4  | 1.5                           | 0.5              | 125.9                  | 5851.2              | 15.97                   | 4.24                     |
| 5  | 1.0                           | 4.0              | 125.6                  | 5461.1              | 13.64                   | 3.63                     |
| 6  | 2.0                           | 3.0              | 125.7                  | 5766.4              | 10.32                   | 4.40                     |
Figure 5. Antibacterial test of papers: without addition (A); with 2% of nanocellulose addition (B); with 2% of nanochitosan addition (C); with 1% of nanochitosan and 1% of nanocellulose addition (D)

4. Conclusions
Rice straw nanocellulose and nanochitosan are potential materials that can be added to the pulp slurry to improve burst index of paper. The mixture of nanocellulose and nanochitosan at a suitable ratio provides positive influences on some mechanical, barrier, and antibacterial properties of paper, leading to potential applications in food and water-resistant packaging.

The antibacterial features on *E. coli* clearly showed in papers with 2% of nanochitosan or with the mixture of 1% nanocellulose and 1% nanochitosan addition. The combination of nanocellulose and nanochitosan in paper demonstrates their efficiency in creating antibacterial ability in papers.

Modified limited hydrolysis method of cellulose for nanocellulose production from various lignocellulosic biomass sources is our know-how [15,16]. Nanocellulose fabrication Using nanocellulose and nanochitosan as additives for the wet part of the paper is a new investigation in this field, so the findings contribute significantly to the development of antibacterial packaging technology from fiber sources and nanomaterials of Vietnam.

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