

Chitosan-Based Coating Application to Enhance Antimicrobial and Water Vapor Barrier Properties of Industry-Manufactured Paper

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<https://doi.org/10.14710/jksa.27.11.515-522>

Article Info

Article history:

Received: 28th July 2024

Revised: 15th November 2024

Accepted: 18th November 2024

Online: 30th November 2024

Keywords:

Chitosan; Antimicrobial; Coated Paper; *S. aureus*; *E. coli*

Abstract

Chitosan, a renowned natural polymer for its wide application, was exploited for industry paper coating solutions. This research aimed to investigate the antimicrobial and water vapor barrier of chitosan solution-coated industry-manufactured paper. The papers were obtained from a national company in Indonesia. The commercially available chitosan with three molecular weight levels (low, medium, and high) was solubilized in sodium acetate buffer and subsequently utilized as the coating solution. The other variable studied was the chitosan concentration in the coating solution, i.e., 1.0, 1.5, and 2.0% (w/w). The antimicrobial activity study was performed by testing against Gram-positive bacteria, represented by *Staphylococcus aureus*, and Gram-negative bacteria, represented by *Escherichia coli*. The low molecular weight chitosan showed the best antimicrobial activity and water vapor barrier performance. The 1 %w low molecular weight chitosan-coated paper had shown good antimicrobial activity, against both *S. aureus* and *E. coli*, with a growth reduction of > 95 %. The most effective antimicrobial activity against *S. aureus* was achieved by paper coated with a 1.5% solution of low molecular weight chitosan. For low molecular weight chitosan-coated paper the most effective water vapor barrier was exhibited at 1 %w chitosan concentration. Having shown the best water vapor barrier while maintaining good antimicrobial activity, the 1.0% solution of low molecular weight chitosan was appointed as the best coating solution in this research.

1. Introduction

Food packaging contributes to food safety as it can retard product deterioration, retain the beneficial effects of processing, extend shelf-life, and maintain or increase the quality and safety of the food [1]. Food packaging markets continuously grow as customers are more concerned with health and food safety. The market value was USD 303.36 billion in 2019 and is predicted to grow by USD 60.33 billion between 2022 and 2025 [2]. Packaging material selection criteria set by the food processing industry include several factors like thermal adhesion, processability, printability, strength, barrier properties (water, oil, and gas barrier), cost-effectiveness, sustainability, and legal requirements [3]. Plastic has been preferably used as food packaging for several reasons connected to its properties, such as flexibility, lightweight, susceptibility against hot or cold weather

without affecting the food content, and low production cost.

The worldwide plastic consumption for food packaging, including polyethylene terephthalate (PET), polyvinyl chloride (PVC), polystyrene (PS), polypropylene (PP), high-density polyethylene (HDPE), and low-density polyethylene (LDPE), were reported up to 20% of its production [2]. However, its beneficial characteristics as food packaging are challenged by the current issue of declining petroleum reserves and the environmental problems arising from its disposal [4]. The depletion of petroleum reserves has disturbed the sustainable use of plastic packaging [5]. Plastic has a very serious negative impact on the environment [2, 6]. Solid waste accumulation at landfills and microplastics in the sea prove to be an environmental disadvantage of plastic

packaging [7]. About 8 million tons of plastic end up in oceans annually, causing serious damage to marine life [2]. These realities urge studies on the alternatives of sustainable and environmentally friendly food packaging materials that benefit health.

Paper and paperboard were the earliest and most widely used materials for some food products, such as milk and its derivatives, beverages, confectionery, and bakery. They cover 31% of the global packaging market segment [3]. Nevertheless, other packaging materials such as glass and metal had been known for having excellent physical, gas and water barrier properties. Plastics are also prominent packaging materials because of their flexibility, size and shape variability, thermal stability, and barrier properties. Still, their petroleum-dependent production and environmental impact are yet considered to be their drawback for future utilization [8, 9]. Despite its biodegradability and abundantly dependent on raw materials, paper still has low barrier properties and, therefore, needs sizing or coating material to improve its properties [9, 10].

In the industrial paper sizing process, certain coating substances, such as starch, casein, and alum, are added to enhance appearance, barrier, and strength [3]. The incorporation of coating materials to paper is commonly performed since cellulose fibers as paper raw material are microporous with a pore size of 0.1–3 Å, causing its limitation in permeability gases and water vapor, famously known as barrier properties [10, 11]. Nonetheless, more valuable food packaging material can be created with the introduction of functional materials having antimicrobial and antioxidant activities [10].

Chitosan is a biopolymer synthesized from chitin, a natural biopolymer constructing crustacean shells with the structural name 2-acetamido-2-deoxy-D-glucopyranose linked by β 1,4-glycosidic bonds through deacetylation reaction. It is merely a copolymer of 2-amino-2-deoxy-D-glucopyranose and 2-acetamido-2-deoxy-D-glucopyranose with a deacetylation degree greater than 50% [12, 13]. The antimicrobial activity of chitosan has been well-known against Gram-positive and Gram-negative bacteria, as well as fungi such as *Candida* species [14, 15]. Tanpichai *et al.* [16] incorporated low (25 kDa) and high (2100 kDa) molecular weight chitosan into steam-exploded bamboo fiber and nanofibrillated cellulose-originated paper. The antimicrobial activity of the paper against *S. aureus* and *E. coli* did not improve despite having been coated with chitosan. However, Cabañas-Romero *et al.* [17] reported that bacterial cellulose-chitosan paper displayed antimicrobial activity, growth inhibition, and killing effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. Prasetyo *et al.* [18] also reported the inhibiting behavior of chitosan-coated oil palm empty fruit bunch (OPEFB) paper against *S. aureus* and *E. coli*.

The investigation of the chitosan-coated paper barrier and antimicrobial activity properties is still challenging to be performed. The incorporation of chitosan coating into industry-made paper has not been

widely investigated. In the effort to participate in the global challenge of using renewable natural resources, this research aimed to make use of chitosan as a seafood waste-originated chemical and to investigate the effect of molecular weight and chitosan concentration on the barrier and antimicrobial activity of chitosan coating on industry made paper. This work discusses chitosan-coated paper antimicrobial activity and water vapor transmission rate (WVTR). The coated paper grammage and characterization, including FTIR and XRD spectra and surface SEM images, are also elaborated.

2. Experimental

2.1. Materials

The low (#448869), medium (#448877), and high (#419419) molecular weight chitosan and sodium acetate used in this research were purchased from Sigma Aldrich, USA. The molecular weights of chitosan obtained from other work by Panda *et al.* [19] were 50–90, 190–310, and 310–375 kDa for low, medium, and high molecular weights, respectively. Glacial acetic acid was purchased from Merck, USA (#695092). The 35 gsm paper was supplied from a national paper manufacturing company. Three coating solutions of chitosan with concentrations of 1.0, 1.5, and 2.0 (%w) were prepared by dissolving corresponding amounts of chitosan in $\text{CH}_3\text{COOH}/0.2 \text{ M CH}_3\text{COONa}$ buffer solution. One hour of stirring using a magnetic stirrer was required to completely dissolve chitosan.

2.2. Coating Process

The equipment used for paper coating is shown in Figure 1. The paper being coated was cut into $10 \times 12 \text{ cm}$ before coating. The paper was then mounted on the glass-made base. A 6–8 mL chitosan in buffer solution (0.3 M $\text{CH}_3\text{COOH}/0.2 \text{ M CH}_3\text{COONa}$) was then pipetted alternately onto the upper and lower side of the paper and spread using a 710 g stainless-steel bar. Afterward, the coated paper was dried using a hot air stream at 60°C to avoid excessive heating. The paper thickness before and after coating was measured using a digital thickness gauge (Mitutoyo, UK).

2.3. Analysis Methods

The antimicrobial analysis was performed against Gram-positive *S. aureus* and Gram-negative *E. coli* aseptically within a laminar airflow safety cabinet and using sterilized equipment. The procedure involved the standard aerobic plate count (APC) method developed by the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA) [20].

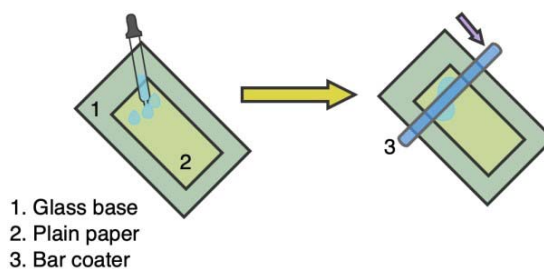


Figure 1. Bar coating process

A bacterial suspension was prepared by incubating nutrient broth (NB) media inoculated with a loopful of bacterial colonies for 24 h at 37°C. Coated paper in the amount of 0.5 g was immersed within 100 mL suspension containing 10^5 cells/mL for each strain, and this was subsequently followed by shaking at 130 rpm and room temperature for 1 h. The concentration of the bacteria within NB media was determined by measuring the optical density (OD) of the suspension and then converting it into cell concentration (cells/mL) using a prepared calibration curve (OD versus APC). By calculating the number of colonies of cell suspension with no contact and after contact with the coated paper, antimicrobial activity can be observed and evaluated.

The paper and chitosan characterizations were carried out using a Fourier Transformed Infrared (FTIR) analyzer (Shimadzu IR Tracer 100, Japan) with scanning wavenumber $400 - 4000 \text{ cm}^{-1}$ for a resolution of 4 cm^{-1} . The measurements were performed under ATR (attenuated total reflectance) mode (a single reflection ATR accessory with a diamond crystal, QATR 10, Shimadzu). The X-ray Diffraction (XRD) instrument was X'Pert Pro (PANalytical, BV, Netherlands). The instrument was operated using $\text{CuK}\alpha$ with an electrical current of 40kV and 30 mA. The crystallinity of the samples can be computed from an XRD diffractogram using Equation (1).

$$\%CrI = \frac{(I_{200} - I_{am})}{I_{200}} \times 100 \quad (1)$$

Where, I_{200} is the maximum intensity of the 200 diffraction plane, and I_{am} is the minimum portion scattered intensity. The paper surface characteristic was analyzed using the scanning electron microscope (SEM) EVO®LS 10 (Carl Zeiss Micro Imaging GmbH, Göttingen, Germany).

The water vapor transmission rate (WVTR) was calculated according to ASTM E-96-80 and similar to the upright cup method by Kim [21]. In this work, the sample specimens were circle cut of uncoated and coated papers with 11 cm diameter. The specimen was mounted and adhered using adhesive tape on the perimeter of a Petri dish containing silica gel where the space between the silica gel and the paper was 5 mm. The amount of water vapor absorbed onto the paper was estimated as the paper mass changes during 24 h storage within a controlled atmosphere container. The relative humidity outside the petri dish was kept at $75 \pm 2\%$, using 100 mL sodium chloride solution (35% w/v), while that inside was 0 % because of the silica gel. The WVTR value was calculated using Equation (2).

$$WVTR = \frac{W}{A \times t} \quad (2)$$

Where, W/t (g.h^{-1}) is the slope obtained from the plot of the sample paperweight increase measured versus time, and A (m^2) is the area of the sample paper. According to ASTM E-96-80, the water vapor permeability (WVP) can also be obtained from WVTR using Equation (3) [22, 23].

$$WVP = \frac{WVTR \times L}{S \times (R_2 - R_1)} \quad (3)$$

Where, L is the paper thickness (m), S is the water vapor pressure at 30°C (4246.69 Pa), and $(R_2 - R_1)$ is the relative humidity difference between the outside and inside of the petri dish.

2.4. Statistical Analysis

The experiments in this work were carried out in duplicate. The measurement and calculation were reported from the average values. To evaluate the difference between %growth reduction values, statistical t-tests were carried out. The effect of chitosan molecular weight and concentration were separately analyzed using one-way ANOVA. Matlab software (R2021b version) was utilized to assist with statistical calculation, including the t-test and ANOVA calculation.

3. Results and Discussion

3.1. The Coated Paper Characteristics

The grammage of the uncoated and coated paper is presented in Figure 2. The uncoated paper, which was obtained from the national paper industry, had a grammage of $35.83 \pm 0.41 \text{ gsm}$ (g.m^{-2}). The addition of coating solution increased the paper grammage to the vicinity of $43 - 50 \text{ gsm}$ (g.m^{-2}) with the percentage of standard deviations (%SD) in the range of $0.8 - 4.75$.

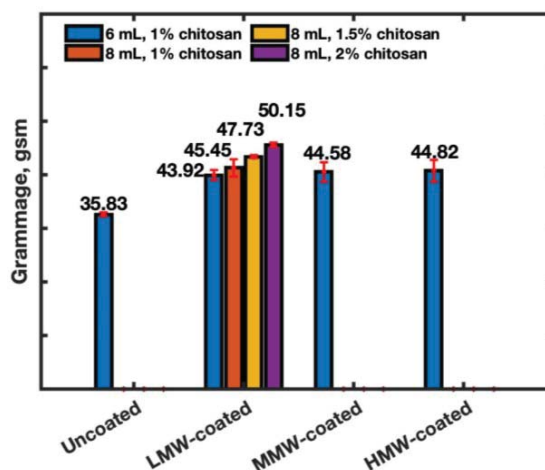


Figure 2. The grammage of studied paper

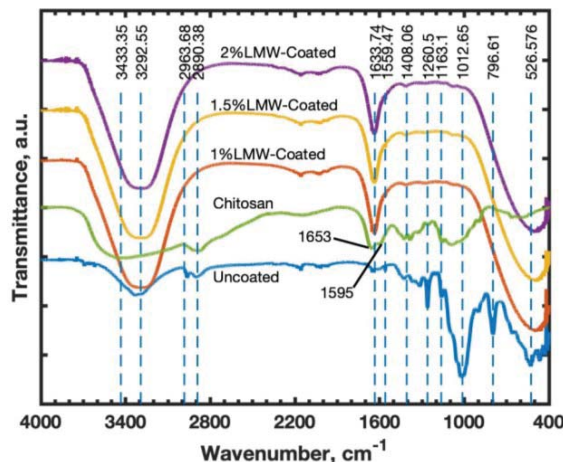


Figure 3. The ATR-FTIR spectra of studied paper and chitosan

The 1% LMW chitosan coating solution affected the grammage of the paper as the increase in volume would cause an increase in coating solution weight. At the same volume and chitosan concentration of coating solution, the grammage of the coated papers also increased as the molecular weights of chitosan used were larger. The molecular weight of macromolecules such as chitosan could elevate the coating solution density and enhance the coated paper's weight.

Figure 3 shows the ATR-FTIR spectra of chitosan as well as the low molecular weight chitosan-coated papers with various chitosan concentrations in the coating solution. The broad band at 3292.55 cm^{-1} represents the O-H stretching. The LMW Chitosan exhibits distinctly a broader band at 3433.35 cm^{-1} , showing the O-H and N-H stretching [17, 24]. Very small peaks at 2963.68 and 2890.38 cm^{-1} only appear in the spectra of uncoated paper. The former may be associated with the axial deformation of the CH_2 group [25]. The latter, which also appears in chitosan spectra, is attributed to aliphatic C-H stretching vibration [26, 27, 28].

The bands at 1653 and 1595 cm^{-1} , respectively, show the presence of chitosan amide I [29] and chitin or chitosan amide II [29, 30]. All the coated papers exhibit sharp and intense bands at 1633.74 cm^{-1} and very weak bands at 1559.47 cm^{-1} , which represent the C=O stretching of amide I and N-H deformation [26]. In this work, the cellulose-chitosan interaction exhibited by all the coated papers resulted in a sharp band at 1633.74 cm^{-1} . The other noticeable bands shown in the uncoated papers are 1260.5 , 1163.1 , and 1012.65 cm^{-1} , representing CH deformation [31], C-O-C stretching [27], and C-O stretching [28], respectively.

Hong *et al.* [32] mentioned that the region of $1500 - 1200\text{ cm}^{-1}$ was considered as local symmetry, which included deformational vibrations, CH_2 groups, and numerous C-OH deformations, while the region of $1200 - 800\text{ cm}^{-1}$ was called the "fingerprint region". It has been reported that CH deformation was assigned at 1267 cm^{-1} for spruce cellulose after 5-minute milling [31]. The bands of 796.61 and 526.576 cm^{-1} on the uncoated paper may be attributed to the additive of the paper, i.e., kaolinite, as it is one of the major papermaking filler components [33]. It has been reported that 540 cm^{-1} corresponded to Si-O-Si bending vibration, while the bands in the range of $780 - 798\text{ cm}^{-1}$ corresponded to Si-O-Si inter tetrahedral bridging bonds in SiO_2 [34].

The XRD spectra of chitosan and paper (uncoated and coated) are presented in Figure 4a and 4b, respectively. Figure 4a shows that two crystalline peaks at 9.04° and 20.06° represent the planes of (020) and (200) HMW chitosan. The 200-plane occurred at the same angle for MMW and LMW chitosan, while the (020) plane shifts to $\sim 10^\circ$. Crystallinity values were 58.54%, 60.04%, and 70.02% for LMW, HMW, and MMW chitosan, aligning with Podgorbunskikh *et al.* [35], who reported peaks at $\sim 10^\circ$ and 20° . Crystallinity increased with molecular weight, but values differed, with 63%, 77%, and 80% for LMW, HMW, and MMW, respectively.

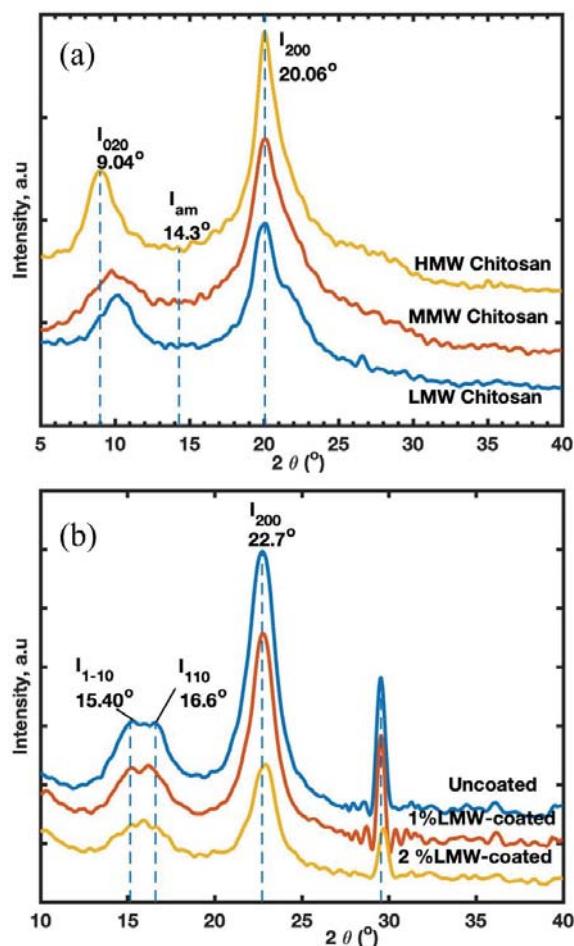


Figure 4. The XRD spectra of (a) chitosan and (b) studied paper

Pongchaiphon *et al.* [27] utilized chitosan with higher molecular weight (540 kDa) and reported almost the same peaks, 9.5° and 19.74° for (020) and (200) planes, respectively, but lower crystallinity of 52.6%. The uncoated and chitosan-coated paper diffractograms are shown in Figure 4b. All the uncoated and coated papers exhibited (200) plane peaks at 22.7° . Another two peaks, (1-10) and (110) planes, appeared at 15.4 and 16.6° . Coating with the chitosan solution caused these two peaks to approach closer, and the chitosan concentration increased. The chitosan coating also caused crystallinity to decrease. The crystallinity values were 77.5, 75.43, and 65.98% for uncoated, 1% for chitosan-coated, and 2% for chitosan-coated papers, respectively. The crystallinity index of raw pulp was reported to be around 70 – 73% [36, 37, 38, 39].

Vehviläinen *et al.* [39] reported the 2 θ positions for bleached softwood Kraft pulp at 17° , 19° , and 26° for (1-10), (110), and (200) crystalline planes, respectively, and peak at 29.54° as exhibited in Figure 4b did not exist. This implied that the uncoated industrial paper used in this work had been added with certain additives that caused this peak to appear. Meanwhile, another work that used plain paper did not report the peak but confirmed that chitosan caused the peak at 29.86° [26].

3.2. Antimicrobial Activity

The antimicrobial activities of the chitosan-coated papers in this work were evaluated against Gram-positive *S. aureus* and Gram-negative *E. coli*. Figure 5 shows the reduction of bacteria growth by 1 %w chitosan-coated papers with various chitosan molecular weights, where each data is presented along with the SD value. The figure depicts that chitosan coating increases the paper's activity against bacteria. It also shows the higher destruction ability of the coated paper against *S. aureus* compared to *E. coli*. There were no differences (p -value = 0.320 and 0.515 for *S. aureus* and *E. coli*, respectively) in the antimicrobial activity of LMW, MMW, and HMW. However, the numerical values for LMW were the highest among the three samples. The experiments were carried out in duplicate and resulted in a %SD in the range of 0.25–0.65.

Cabañas-Romero *et al.* [17] also reported the growth inhibition by bacterial cellulose-chitosan paper against *S. aureus* and Gram-negative *P. aeruginosa*. According to that work, the bacterial cellulose-chitosan paper inhibited the growth and showed bactericidal activity. This may explain why the increase of chitosan concentration from 1 to 2% did not affect the %reduction of *S. aureus* in Figure 6 (p -value = 0.381) and resulted in almost 100% reduction. The antimicrobial activity of chitosan-coated paper results from the electrostatic interaction of the positive amino group of the attached chitosan on the paper surface and the negative charge on the surface of the microbial cell, which changes cell membrane permeability and leads to cell death [15]. The lower growth reduction by HMW chitosan was also shown as higher molecular weight, which resulted in a higher viscosity coating solution and hindered the mobility of chitosan to be appropriately attached to the surface [16].

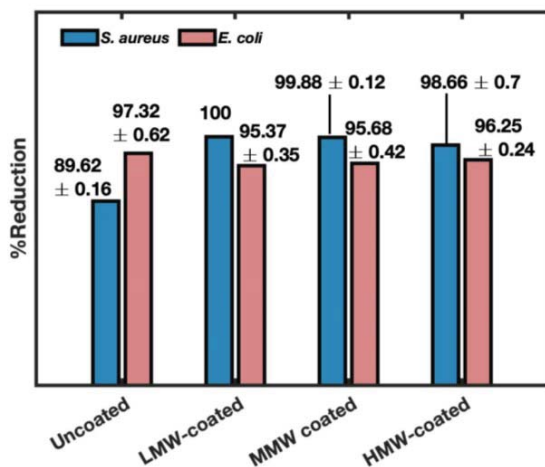


Figure 5. The %reduction of *S. aureus* and *E. coli* growth of 1 %w chitosan-coated paper

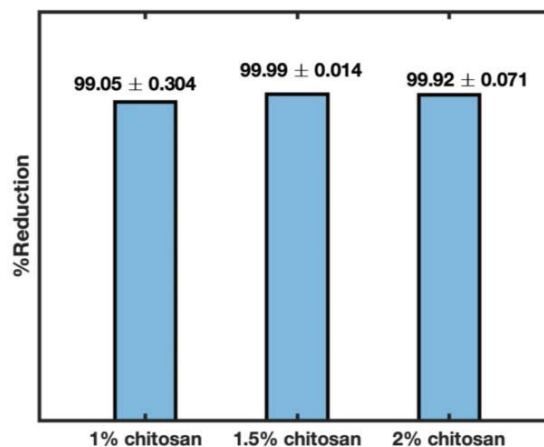


Figure 6. The %reduction of *S. aureus* growth of low molecular weight chitosan-coated paper

3.3. Water Vapor Transfer Rate

The water vapor transfer rates and water vapor permeability of the uncoated and chitosan-coated paper are presented in Figures 7a and 7b. The figure describes that the paper coating with chitosan drastically reduces the water vapor transfer rate from 2.34 to below $2 \text{ g.m}^{-2}.\text{h}^{-1}$ (Figure 7a) with %SD around 3.6 – 7.6, while the water vapor permeability from 4.506 to $0.289 \times 10^{-8} \text{ g.m}^{-1}.\text{h}^{-1}.\text{Pa}^{-1}$. The very low water vapor transfer rates were yielded after paper coating because the hydrophobic chitosan particles within the coating solution filled the pores amidst the intertwined paper fibers and, as a result, blocked the water vapor transporting through the paper.

The one-way ANOVA resulted in very low p -values (1.39×10^{-5} and 4.03×10^{-5}) for the effect of chitosan molecular weight and concentration, respectively. These low p -values (< 0.05) show that the chitosan molecular weight and chitosan concentration significantly affect the water vapor transfer rates and water vapor permeability of the coated papers. This is also supported by the surface SEM image of the coated paper shown in Figure 8. From the figure, it can be seen that the paper pore is filled with chitosan-coated paper. Figures 8a and 8b also show that increasing the chitosan concentration lessens the pore existence and, therefore, leads to a smoother paper surface. The paper surface pore filling by chitosan was promoted by hydroxyl group interaction between chitosan and paper cellulose fibers [16, 40].

Furthermore, the hydrophobic characteristic of chitosan-coated paper was also evidenced by the increase in contact angle molecular weight and concentration of chitosan [16]. This hydrophobic characteristic could support the chitosan coating to repulse water molecules from the coated paper surface. Nevertheless, the coating material is not perfectly evenly distributed on the surface, which may be caused by the use of a manual hand-slide bar coater during the coating process.

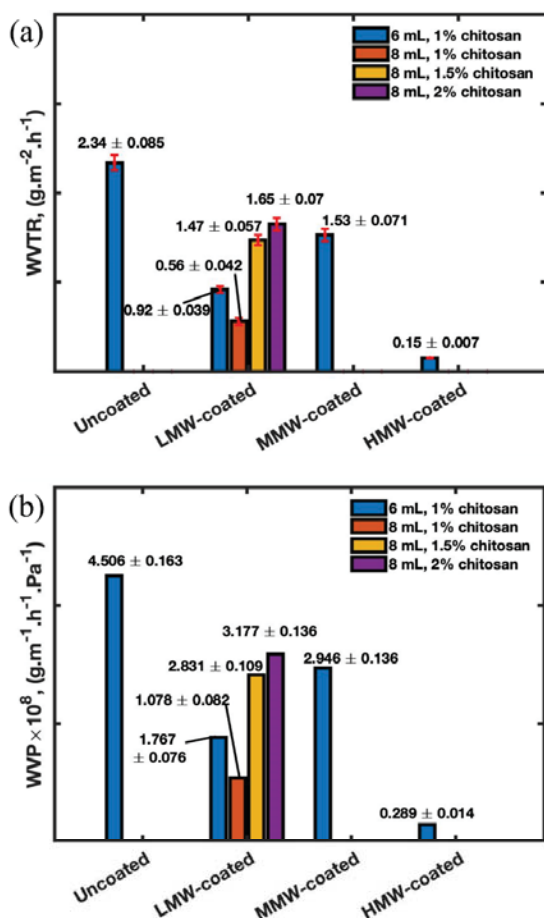


Figure 7. (a) The water vapor transfer rate (WVTR) and (b) The water vapor permeability (WVP) of the studied paper

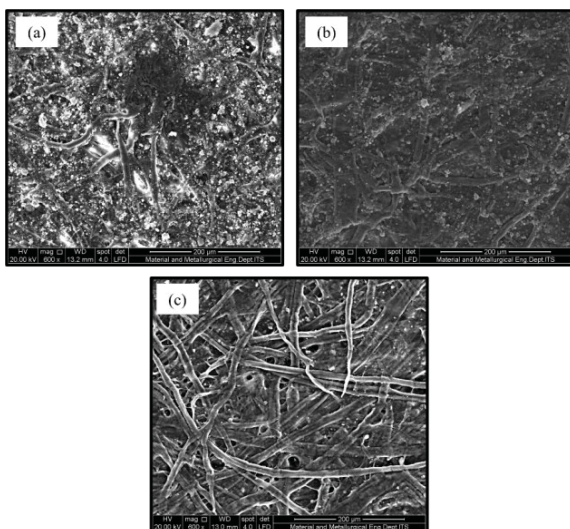


Figure 8. The surface SEM images of (a) 1% LMW chitosan-coated, (b) 2% LMWchitosan-coated paper, and (c) uncoated paper

4. Conclusion

A study on the chitosan coating on industrially manufactured paper has been performed in this research. The FTIR and XRD spectra of the coated papers revealed the chitosan-cellulose interaction. Antibacterial

activities against *S. aureus* and *E. coli* have been shown to have a growth reduction of >95%. Low molecular weight chitosan exhibited the highest antibacterial activity, whereas the activity against *S. aureus* was higher. A low molecular weight chitosan concentration of 1% has reduced almost 100% of *S. aureus* growth. The chitosan coating has also reduced the water vapor transfer rate significantly to below $2 \text{ g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ by filling the pore between paper cellulose fiber networks. Despite the high antimicrobial activity potential shown by the chitosan-coated paper in this work, a rigorous study should be carried out to elaborate on the impact of chitosan-coated industry-manufactured paper application as a food wrap or packaging on the food shelf life and safety. The biodegradability and mechanical properties of the chitosan-coated paper must be thoroughly studied in future work.

Acknowledgment

The authors acknowledged the support of Dyah Ayu Ambarsari, the technical staff of the Polymer Laboratory at the Chemical Engineering Department of Universitas Surabaya.

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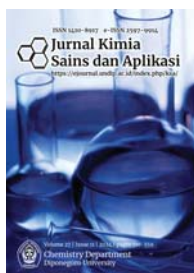


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