Preparation and preliminary characterization of sago flour and semi refined kappa carrageenan-based biocomposite film incorporated with coconut crabs chitosan nanoparticles

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Abstract. The use of nano-sized chitosan natural polymers served as biopolymers containing antimicrobial substances is promising to be utilized as preservatives that could maintain quality and extend the shelf-life of foods. The objective of this research was to evaluate the effect of chitosan coconut crabs nanoparticles (CsCC NPs) incorporation to the sago flour (SF) and semi-refined kappa carrageenan (SR KC) based biocomposite film on their mechanical and barrier properties. The CsCC NPs suspension was prepared by the beads-milling method. The CsCC NPs was incorporated with various concentration (0, 1, 2, 3%) and introduced into 2 g of SF and SR KC (2:1) with the addition of Carboxymethyl Cellulose (CMC) and glycerol (2:1) as a plasticizer. The results showed that the brightness value ($L^*$) of prepared biocomposite film was at the range of 59.21-60.44. The thickness, tensile strength, and elongation at break of the films were at the range of 0.082-0.172 mm, 3.22-8.09 MPa, 18.49-24.25%, respectively, while the water vapor permeability (WVP) of the films were at a range of 3.61-7.99 gm/m² h⁻¹ kPa⁻¹. It is indicated that the incorporation of CsCC NPs on the investigated biocomposite film could improve the films’ properties and have enormous potential to be developed as biodegradable active packaging.

1. Introduction

Nonbiodegradable polymers come from plastic-based synthetic polymers that are widely used in food packaging because the production costs are relatively cheap, durable, water resistant and can be consumed directly. The use of nonbiodegradable plastic packaging will cause problems with environmental damage, so as to encourage the development of biodegradable and environmentally friendly materials, especially food packaging [1, 2]. Other applications of materials that have a nanoparticle size function as antimicrobials mainly prevent bacterial growth [3]. The use of...
nanotechnology is currently attracting attention and has a major influence on the food packaging industry for the development of food packaging in ensuring product quality and food safety [4].

The potential of chitosan to act as a food preservative that comes from nature. Chitosan is also an excellent film-forming material [5]. Chitosan film has a permeability to gas properties [6]. Polymer nanotechnology is an area that dynamically develops materials loaded with special particulates on the nanoscale [7]. Nano-bio-biopolymers are extensively studied earlier for food packaging applications [8]. Chitosan film loaded with cellulose nano-crystals improves the permeation properties of films. Nano-bioccomposite is a new biopolymer matrix applying physical-chemical interactions [9]. The use of chitosan particles as fillers in the manufacture of biocomposite films can improve mechanical properties and barrier in films [10, 11]. The use of chitosan as a packaging material has the advantage of being an antibacterial activity [12]. The addition of chitosan nanoparticles as a filler in the manufacture of films using carrageenan kappa and tapioca starch showed the ability of accessibility and antimicrobial properties [13]. In filmmaking research using filler nanoparticles to improve the film's ability to significantly resist water vapor permeability [14].

Edible films have emerged as alternative packaging for synthetic plastic packaging in food applications. The development of seaweed-based biopolymers for edible films[15]. Characterization of novel composite semi-refined iota carrageenan based edible film [16]. Mechanical and barrier properties of semi-refined kappa carrageenan-based composite edible film and its application [17]. Edibility and biodegradability are the most beneficial properties of edible films and edible coating that can be eaten [18]. Edible nano-biocomposite film is a new application for food packaging. The layer of the adenine polysaccharide nanoparticles serves as an active molecular inhibitor [19]. The use of nano-sized natural polymer chitosan serves as a biopolymer which has antimicrobial properties so it can be used as a preservative that extends the shelf life of foodstuffs. Therefore this study aims to determine the mechanical properties and barrier properties of sago flour (SF) films substituted by kappa carrageenan (KCRG) with filler chitosan coconut crabs nanoparticles (CsCC NPs) as a result of the beads-milling method. The novelty of this is research was to develop biocomposite films sago flour-kappa carrageenan semi-refined as matrix and then CsCC NPs suspension as fillers.

2. Experimental

2.1. Materials

Chitosan coconut crabs shell is the result of chitin deacetylation process, which is then processed using the beads-milling method to produce chitosan nanoparticles, which are used as fillers in films (data not shown) conducted at the Laboratory of Nanotechnology and Graphene Research Center, Padjadjaran University. Sago flour is obtained from the traditional market of Ternate City, while semi-fine kappa-carrageenan is supplied by Galic Artabahari, Co., Ltd. (West Cikarang, Indonesia), these two materials are used as the main components of the film matrix. Carboxymethyl cellulose (CMC) and glycerol are used as plasticizers and purchased from Brata Kimia (Surakarta, Indonesia). Aquadest is used for all sample preparations. As well as chemicals to be used as the analytical material.

2.2. Methods

2.2.1. Preparation of Bio-nanocomposite Film

The edible film made in this study is a film made from chitosan nanoparticle products as the filler with the main raw materials, namely sago flour (SF) and kappa carrageenan (KCRG). The solvents used were distilled water, the use of the main ingredients was the concentration of sago flour and carrageenan, while the chitosan coconut crabs nanoparticles (CsCC NPs) with a certain concentration only as a filler with plasticizers used glycerol and carboxyl methyl cellulose (CMC) (2: 1) [20]. The procedure for making films was modified from Harris's (1999) method. While the formulation of the material used refers to research [25]. Briefly the procedure for making edible films is as follows, the main ingredients of sago flour and carrageenan kappa (2: 1 b/v) are dissolved in 100 ml of distilled
water and add glycerol and CMC (2: 1 v/v), then add 0% CCCsNPs concentration, 1%, 2%, 3% (v/v) results from the beads-milling (BM) method. The process of mixing the solution using a heating magnetic stirrer at 1500 rpm to a temperature of 65 °C is maintained for 10 minutes. Furthermore, the temperature is lowered to 45 °C, then the printing process on the plastic plate size (W x L x H: 15 x 23 x 2 cm) is evenly distributed and cooling is done at room temperature for 20 minutes, and to get the film followed by drying using cabinet drying for 12 hours at a temperature of 60 °C with relative room humidity. After cooling to room temperature, each dry film is carefully peeled from the casting surface into an aluminum foil packaging to avoid hygroscopicity and storage in a plastic box containing silica gel (0% RH) at 28 ± 2 °C for 24 hours before analysis [21].

2.2.2. Color Measurements
Color values (L*, a*, b*) of the film were measured with CR 310 Minolta Chroma Meter (Minolta Camera Co., Ltd.). The film is placed on a standard white plate (calibration plate) and the Hunter Lab color scale is used to measure color [22]. Each film sample is measured in four different reading positions. The total color difference (ΔE) is calculated as:

\[ \Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \]  

(1)

2.2.3. Film Thickness (FT)
Measurement of film thickness with an accuracy rate of 0.001 mm using a digital micrometer 0-25 mm (Krisbow, Indonesia). The sample is measured randomly at four different points, then the average thickness is calculated.

2.2.4. Tensile Strength (TS) and Elongation at Break (E)
Tensile strength (TS) and elongation (E) are measured by Universal Testing Machine (Zwick I Model Z0.5, UK) with the standard method ASTM D882-00 (ASTM, 2000) [23]. The Edible Film (EF) samples are cut in rectangular strips with a length of 100 mm and a width of 25 mm using a printing device, measured by the thickness of the film with a micrometer, then mounted on the clamp on the tool. The tool is set with a pre-load value of 2 N/mm², a test speed of 10 mm/minute, and a clamping distance of 50 mm. The start button is pressed 2 times where the 1st pressure activates the tool and the 2nd pressure will operate the tool so that the test takes place.

2.2.5. Water Vapour Transmission Rate
Testing of water vapor transmission rate(WVTR) levels in film samples was carried out gravimetrically following the ASTM E96-00 (ASTM, 2000) method with modifications [24]. 10 grams of silica gel is placed into the Petri dish. The film sample was cut round and glued to the top surface of a 6 x 6 cm 2 Petri dish using plasticine. Every 1 hour for 8 hours of testing, weight gain from the Petri dish was measured. The weight change of silica gel (g) in the Petri dish is recorded as a function of time and the slope is calculated using linear regression (change in weight vs. time). Slope (g/hour) divided by the area or surface area of the petri dish (m²) is the rate of water vapor transmission (WVTR). The film thickness is measured by a digital micrometer (Digital Outside Micrometer, Inside, Germany) with an accuracy of 0.0001 mm. Then the Water Vapor Permeability (VWP) value with units of g.mm/m².KPa.jam can be calculated using the following formula:

\[ VWP = \frac{WVTR \times x}{\Delta P} \]  

(2)
in which x (mm) is the film thickness and ΔP (Kpa) is the air pressure.

2.3. Statistical Analysis
The data were analyzed statistically by one-way variance analysis (ANOVA) at a significance level of 0.05 and the difference in mean values was determined by Duncan's Multiple Range Test (DMRT) (p <0.05) to determine the difference between concentration treatments. For statistical analysis using the SPSS 16 program.
3. Results and Discussion

The characterization of the bio-nanocomposite edible film properties with the chitosan coconut crabs (CsCC NPs) nanoparticles suspension by the beads-milling method with an average diameter (D10) of ±75 – 528 nm that applicable as nano reinforcement for biocomposite films is presented in Table 1. Each parameter showed a certain tendency as the concentration between refined kappa semi-carrageenan sago flour and chitosan nanoparticle concentration as filler. The effect of using chitosan nanoparticles for each edible film is discussed separately.

Table 1. Characterization of mechanical and barrier of nano-biocomposite edible film properties of semi refined kappa sago-carrageenan flour with coconut crabs chitosan nanoparticle filler.

| Film       | CsCC NPs (% v/v) | Color (L*) | Thickness (mm) | Tensile Strength (MPa) | Elongation (%) | WVP (g mm/m² h⁻¹ kPa⁻¹) |
|------------|------------------|------------|----------------|------------------------|----------------|-------------------------|
| SF: SRKC (2:1) | 0                | 59.61 ±0.13 | 0.14 ±0.02 | 5.11 ±0.38 | 24.25 ±2.25 | 3.61 ±1.07 |
|            | 1                | 59.21 ±0.09 | 0.08 ±0.01 | 3.22 ±0.31 | 23.17 ±0.24 | 3.98 ±0.55 |
|            | 2                | 59.54 ±0.44 | 0.12 ±0.01 | 7.57 ±0.08 | 18.49 ±0.64 | 7.99 ±1.71 |
|            | 3                | 60.44 ±0.58 | 0.17 ±0.02 | 8.10 ±0.09 | 24.13 ±0.28 | 2.61 ±1.07 |

Note: Value in the same line followed by a different letter was significantly different (p < 0.05).

3.1. Color Measurements

Color values in edible films are the most important factor in the appearance of food products and overall are an assessment of consumer acceptance of the product [27]. The color properties of the SF-KCRG nano-biocomposite film with different concentrations of CsCC NPs according to the system of color values (L*) are shown in Table 1. Brightness color values (L*) with 3% CsCC NPs concentrations show the brightness level is higher than the lower concentration (p <0.05). Color values are indicated that the film has good brightness transparency in addition to being influenced by different CsCC NPs concentrations, also because of the use of SF-KCRG material as a matrix that leads to a transparent formation. The effect of wrapping treatment by composite SRKC film in maintaining the color [17]. Transparency of biocomposite films with nano chitosan as filler is indicated by the value of the sample [28]. On the other hand, the addition of plasticizers such as glycerol in film composites using a number of concentrations can produce better films [29]. In low concentrations, fillers as CSNPs suspension such as carrageenan-tapioca with suspension do not affect the transparency of composite films [30].

3.2. Thickness

The film thickness is affected during the printing process on plastic molds. The uniformity of film thickness is also influenced by the SF-KCRG material which is easily gelatinized due to heat and easily retrogressed in room temperature conditions. Film thickness with CsCC NPs filler concentrations varied between 0.08-0.17 mm as shown in Table 1. From the results of the observations showed that the CsCC NPs concentration was low, then the thickness was obtained 0.08 mm in the film.

The use of concentrations of 0.5% sorbitol plasticizer in semi-refined carrageenan materials showed that the film thickness level was significantly influenced by sorbitol concentration [18]. The thickness of the film is influenced by the wide mold, the volume of the solution, and the total dissolved solids [31]. Drying conditions also affect the thickness of edible films. Higher temperatures and longer drying times will evaporate more water, allowing edible films to make thinner thicknesses [32].
3.3. Tensile Strength and Elongation at Break
The results in Table 1 shows that the tensile strength of films with various concentrations of NPs CsCC ranges from 3.22 to 10.10 MPa. The addition of CsCC NPs 3% (v/v) shows the highest tensile strength compared to other concentrations. However, from the results of the statistical test using the DMRT test (α = 0.05), it was shown that the CsCC NPs concentration used was not significantly different. Elongation at break of edible films with various NPs CsCC concentrations were 18.49-24.25%, respectively (Table 1.). The results show that the higher the concentration of CsCC NPs, the lower the elongation. Elongation of edible films is inversely proportional to tensile strength. The difference in 1% CsCC NPs concentration interval is too low so it does not significantly affect elongation.

The higher concentration of plasticizer sorbitol affects the film thickness level [26]. The plasticizer is usually added to the polymeric matrix to overcome the fragility of the film, because it can reduce this strength, thereby increasing film flexibility and elongation. The mechanical properties with higher tensile strength, as well as percentage elongation, will be preferable for food packaging films [21]. The more compact the edible film matrix, the more force is needed to break the film. This leads to a decrease in the film's ability to change shape when it is subjected to certain strengths so that the film tends to break or lose elasticity [26]. These mechanical and solubility properties suggest that bio-nanocomposite film of semi-refined kappa carrageenan and nanoparticle ZnO can be effectively used as food packaging material [32].

3.4. Water Vapor Transmission Rate (WVTR)
The results in Table 1 shows that the water vapor transmission rate (WVTR) of the SF-KCRG edible film with concentrations of CsCC NPs filler (0, 1, 2, 3%) is around 3.61-7.99 g.mm/m².h.kPa⁻¹. The results showed that WVTR films with an addition of 3% (v/v) NPs CsCC concentration (3.61 g.mm/m².h.kPa⁻¹) were the lowest values compared to other CsCC NPs concentrations. However, the results of the statistical test using the DMRT test (α = 0.05) showed that the various CsCC NPs concentrations used were not significantly different. The lowest WVTR shows that edible film has the best properties. That means water molecules, both from the surrounding environment or from packaged products, do not easily penetrate the film layer, thus, the quality of food products, such as weight, crispness, or freshness, is not affected. Water vapor transmission rates depend on the ratio of hydrophilic and hydrophobic materials in film formulas. WVTR is often used to study the moisture transport through the film and is of interest in almost all research into the characterization of biopolymers because it is a key factor in food packaging to support food against water adsorption and desorption [33].

4. Conclusion
Mechanical at barrier properties of SF-KCRG edible film with concentration of CsCC NPs filler (0, 1, 2, 3%) showed that the brightness color value (L*) with 3% CsCC NPs concentration showed higher brightness level, as well as film thickness and tensile strength, and elongation increased with the addition of 3% CsCC NPs concentration, but the water vapor transmission rate (WVTR) decreased with increasing CsCC NPs concentration. Thus, it can be concluded that the best properties of SF-KCRG edible film with concentrations of 3% CsCC NPs filler for further production in advanced research applications.

References
[1] Spotti ML, CecchiniJP, Spotti M J andCarrara C R 2016 J. Food Science and Technology 68 127-134
[2] Sukhija S, Singh S andRear C S 2016 J. Food Hydrocolloids 60 128-137
[3] Pathankot K, Manubolu M andHwang H M 2017 J. of Food and Drug Analysis 25(2) 245-253
[4] Sudibyo A and T F Hutajulu 2013 J. Kimia Kemasan 35(1) 6-19
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