

## Article

# Edible Carrageenan Films Reinforced with Starch and Nanocellulose: Development and Characterization

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**Abstract:** Currently, from the sustainable development point of view, edible films are used as potential substitutes for plastics in food packaging, but their properties still have limitations and require further improvement. In this work, novel edible carrageenan films reinforced with starch granules and nanocellulose were developed and investigated for application as a bio-based food packaging system. The nanocellulose was used to improve film mechanical properties. Aloe vera gel was incorporated for antibacterial properties. Glycerol and sesame oil were added as plasticizers into the nanocomposite film to improve flexibility and moisture resistance. The interactions between charged polysaccharide functional groups were confirmed by FTIR spectroscopy. The migration of starch particles on the upper film surface resulting in increased surface roughness was demonstrated by scanning electron and atomic force microscopy methods. Thermogravimetric analysis showed that all films were stable up to 200 °C. The increase in nanocellulose content in films offered improved mechanical properties and surface hydrophilicity (confirmed by measurements of contact angle and mechanical properties). The film with a carrageenan/starch ratio of 1.5:1, 2.5 mL of nanocellulose and 0.5 mL of glycerol was chosen as the optimal. It demonstrated water vapor permeability of  $6.4 \times 10^{-10}$  g/(s m Pa), oil permeability of 2%, water solubility of 42%, and moisture absorption of 29%. This film is promising as a biodegradable edible food packaging material for fruits and vegetables to avoid plastic.

**Keywords:** starch; carrageenan; nanocellulose; aloe vera; edible film; packaging material



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## 1. Introduction

The growth of the world's population, urbanization, and globalization have led to an increase in food production and demand resulting in exacerbated problems with storage and food safety [1]. As a result, sustainable packaging is becoming a higher priority for both brands and consumers [2]. Packaging materials are useful for extending the life of food and improving its quality during transportation, storage, and distribution [3]. Food packaging uses a variety of materials such as plastic, paper, metal, and glass [4]. The most widely used single-use packaging materials are non-biodegradable plastics, which result in millions of tons of waste, harming the environment and human health. Sustainable packaging involves finding, developing, and using packaging solutions that have minimal impact on the environment [5,6]. Therefore, the food industry is looking for a replacement

for non-biodegradable plastics with environmentally friendly and biodegradable ones [7]. Based on a literature review, it has been shown that biodegradable films are much better suited for food packaging compared to other materials [8]. There are already bio-based edible coatings in the market, used to extend the shelf life, but there is a need to expand basic research in the field of sustainable and biodegradable edible food packaging [9]. It is also very important to use packaging materials that can be obtained from many renewable sources [10]. Edible coatings can consist of lipids, proteins, composites, and, especially, polysaccharides.

Carrageenan (polysaccharide derived from seaweed) is one of the most promising phycocolloids and may be used for blending with starch due to its excellent film-forming ability [10]. The use of this polymer alone for films in packaging area also has limitations, especially with regard to its brittleness, vapor permeability, and water resistance. However, the reinforcement of carrageenan with starch granules, which are also a biodegradable polymer, should result in satisfactory properties for forming a packaging material. Starch is a widely accepted biodegradable material for food packaging [11]. Arrowroot (starch from tropical plants in the family Maranthaceae) has an important role in the development of biodegradable products and materials. The arrowroot rhizome is the main source of starch and fiber with high amylose content widely used in the food industry (for example, for biscuits, puddings, porridge, etc.) and other areas [12]. This resulting starch has many advantages: versatility, environmental friendliness, non-toxicity, bioaccumulation and blood adaptability [13,14]. In this study, the preparation of carrageenan and starch mixtures was carried out at room temperature, so there was no starch solubilization. In this case, the starch grains were preserved and acted as a reinforcement in the carrageenan matrix. Moreover, these polysaccharides (carrageenan and starch) obtained from biomass sources (plants and seaweeds) have significant advantages, including their low cost and abundance in nature. The use of biocomposites consisting of components derived from agricultural waste provides more sustainable packaging, promoting a circular economy [15].

To the best of our knowledge, carrageenan was actively investigated as an additive for starch-based films to improve their properties. The effect of the addition of k-carrageenan into a jicama (*Pachyrhizus erosus*)-starch-based edible coating for grapevines (*Vitis vinifera* L.) was studied [16]. The coating with 2.5% k-carrageenan was the best-selected treatment for grapevines with improved physical characteristics. Thakur et al. have completed in-depth research on rice starch (RS)/carrageenan composite as an edible film [17–20]. The work [17] was devoted to the development of the rice starch/carrageenan film with various formulations (1–4 wt.% rice starch, 0.5–2 wt.% carrageenan, 0.3–0.9 wt.% stearic acid (SA), and glycerol as a plasticizer). The film with the formulation of 2% carrageenan, 2% RS, 0.3% SA, 30% glycerol, and 0.2% surfactant (Tween®20) provided optimal properties. The effect of starches extracted from eight rice varieties on the properties of RS/carrageenan films was also investigated [18]. The film from “Reiziq” starch had minimum thickness, solubility, water vapor permeability, opacity, and better mechanical properties. The effect of different hydrophobic components (butyric, lauric, palmitic, oleic, stearic acids and sucrose fatty acid ester) on the structure and permeability properties of the RS/carrageenan films was investigated in reference [19]. The continuation of this work of the authors was the investigation of the possibility to enhance the shelf life of plum fruit coated with this RS/carrageenan coating blended with sucrose fatty acid ester [20]. To the best of our knowledge, the effect of starch as a reinforced agent for carrageenan-based films was not investigated.

Nowadays, to improve the properties of polymer materials for tailored packaging applications, there has been an increased interest in nanofillers (in 100 nm range) [21]. Nanocellulose (NC) is one such biodegradable renewable nanofiller. The NC introduction into biopolymers allows synergistic improvement in the barrier, thermomechanical, and rheological properties of the material [21]. Nanocellulose has two main geometrical forms: nanocrystals and nanofibrils [22]. Cellulose nanofibrils, as a rule, have flexible structures of a ~50–3000 nm length and ~5–100 nm diameter [23]. They are usually prepared by

defibrillation using mechanical forces [24]. Cellulose nanocrystals have rigid structures and are characterized by ~3–50 nm diameter and ~50–1000 nm length [25,26]. This NC form is usually obtained by extraction from agricultural wastes (rind of cassava, pomelo, walnut, banana peel, straws and stems, etc. [27–31]) by hydrolysis. Nanocellulose has enhanced mechanical properties [22]. In this study, nanocellulose fibers were used as a reinforced agent for carrageenan-based films to improve mechanical properties.

The introduction of other herbs (for example, aloe vera) and additives (for example, oil or glycerol) into edible packaging films allows for the improvement in or provision of new properties, making their use more effective and valuable. Aloe vera (*Liliaceae* family plant) was used for carrageenan-based films to provide antibacterial properties for films. It is widely used in pharmaceuticals, cosmetics, and food products such as drinks and snacks [32]. It has excellent tolerance to drought conditions due to the ability to absorb and retain water for a longer time [33]. Currently, aloe vera gel is attracting great attention from researchers due to its antioxidant and antimicrobial properties, since adding it to packaging materials helps to maintain the safety and quality of products by reducing water loss due to the physical barrier around the product and inhibiting the growth of some microorganisms [34,35]. Glycerol and sesame oil were added into carrageenan-based films as plasticizers. Sesame oil is rich in unsaturated fatty acids and natural antioxidants and has low temperature decomposition [36]. The addition of sesame oil as a plasticizer, compatibilizer, and anti-oxidant into the developed film improves hygroscopic properties [19]. Glycerol is widely used as a plasticizer for the production of films due to its compatibility [37], which results in improved mechanical properties (for example, reducing film brittleness and fragility) by reducing the forces between starch molecules [38].

Thus, the aim of this study was to develop and investigate films from carrageenan reinforced by starch and nanocellulose (nanofibrils) for the prospective development of bio-based edible food packaging materials. The novelty of this study was the investigation of the effect of starch and nanocellulose as reinforcement agents for carrageenan-based films. The properties of the films were optimized by the variation in the carrageenan/starch ratio and incorporation of various concentrations of the second reinforcing agent, nanocellulose. Aloe vera gel was incorporated into the system to provide antibacterial properties for the films. The application of glycerol and sesame oil as plasticizer and anti-oxidant, respectively, allowed the film's mechanical properties, flexibility, and moisture resistance to be improved. The structure and physicochemical properties based on variations in the film composition were studied by various analysis methods: FTIR spectroscopy, scanning electron (SEM) and atomic force (AFM) microscopies, thermogravimetric analysis (TGA), and measurements of contact angle and mechanical properties. To evaluate the prospects of using an edible film as a packaging material, parameters such as water vapor and oil permeability, moisture absorption, and solubility in water for the film with optimal composition were studied.

## 2. Materials and Methods

### 2.1. Extraction of Polysaccharides

Extraction of starch (arrowroot; Kottayam, Kerala, India) was carried out as follows: selected arrowroot rhizomes were peeled, washed, and sliced. The sliced rhizome was then immersed in 0.03 wt.% potassium metabisulfite solution for 15 min, which was later crushed in deionized water in the ratio of 1:2, followed by high speed blending. The homogenous mass obtained was filtered using double cotton cloth. The mass was washed five times to completely remove the starch present. The filtrate obtained was kept for 12 h for the sedimentation of starch, and the water was decanted off carefully. The starch obtained was then dried at 60 °C for 5 h followed with grinding using a mixer [39].

Extraction of carrageenan (Kottayam, Kerala, India) was carried out as follows: algal biomass was collected from Trivandrum (Kerala, India). The algal mixture (air-dried at 60 °C) was refluxed in an extracting medium of NaOH solution in the ratio of 1:20 for 2 h. The hot extract was filtered through a porous glass filter into cold ethanol, resulting in

the precipitation of carrageenan. The precipitated carrageenan was separated from the ethanol–water mixture by filtration, washed (with cold ethanol), and dried at 60 °C [40].

Extraction of nanocellulose (Kottayam, Kerala, India) was carried out as follows: pineapple leaves were collected from the field at Kottayam (Kerala, India). The leaves were chopped into small pieces of 2–3 cm in length and dried under sunlight. Then, the leaves were ground into powder, which was subjected to the alkali treatment using 4 wt.% NaOH at 90 °C for 2 h to remove lignin and hemicellulose with further bleaching using 3 wt.% NaClO<sub>2</sub> at 90 °C for three times for the complete removal of lignin. The bleached sample was washed thoroughly using distilled water followed by acid hydrolysis using 10 wt.% H<sub>2</sub>C<sub>2</sub>O<sub>4</sub> coupled with steam explosion continuously for 3 h with temperature and pressure reaching a maximum of 120 °C and 20 lb. The obtained nanocellulose was thoroughly washed and centrifuged to remove any acid content. The nanocellulose was then homogenized in deionized water at 8000 rpm using a high speed homogenizer. The nanocellulose was then freeze dried and stored [41,42].

## 2.2. Materials

The polysaccharide–carrageenan obtained was used as a matrix for the development of films. Starch, nanocellulose, and aloe vera gel obtained from arrowroot rhizomes, pineapple leaves, and aloe vera plants were used as reinforcing agents and an additive to improve the mechanical and antibacterial properties of the films, respectively. Arrowroot rhizomes and pineapple leaves were collected from the Kottayam locality of Kerala, India, whereas algal biomass was collected from Trivandrum, Kerala, India. Sesame oil (Pazhangadi Oil Industries, Kerala, India) and glycerol (Vekton, St. Petersburg, Russia) were used as an antioxidant and plasticizer for films, respectively. Potassium metabisulphite (Nice chemicals, India), sodium hydroxide flakes (NaOH), and oxalic acid crystals (H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>) purchased from Sigma-Aldrich (St. Louis, MO, USA), sodium chlorite (NaClO<sub>2</sub>) and ethanol from Nice Chemicals (Kerala, India) were of laboratory grade.

## 2.3. Film Preparation

The preparation of films was as follows: the calculated amount of carrageenan and starch was mixed by grinding powders in tracing paper and dissolved in 80 mL of water with constant stirring using a mechanical stirrer at a speed of 500 rpm to obtain a homogeneous dispersion. Carrageenan and starch varied in ratios of 2:1, 1.5:1, 1:1, and 1:2 to obtain the optimal properties. After complete dissolution and the formation of a homogeneous dispersion, the antibacterial agent aloe vera gel (2 mL in 10 mL water) was added and stirred for 30 min. The content of aloe vera gel in the dispersion was ~3 wt.%. After, 2 drops of sesame oil was added for less water absorption with stirring for 30 min. The next step was to add a nanocellulose solution (2.5 mL or 5 mL in water in a ratio of 1:4 to obtain 0.2 and 0.3 wt.% NC in the dispersion, respectively) followed by stirring for 2 h. The nanocellulose suspension used contained 7 wt.% nanofibers. This value was determined as follows: the nanocellulose dispersion of the determined mass was dried at 90 °C for 24 h, then weighed, and a calculation was made based on the ratio of the mass of the solid residue of NC and the mass of the dispersion. Also, to confirm the presence of fibrils, the solid residue of NC was studied by the SEM method. FTIR analysis confirmed that the material was composed only of nanocellulose. After preparing this nanodispersion, films were prepared by pouring onto Petri dishes, followed by evaporation of the solvent at room temperature (25 °C). The optimal composition of the carrageenan/starch (with ratio of 1.5:1) film was prepared according to the method described above, but with the addition of the solution of 0.5 mL glycerol (to obtain 0.6 wt.% in the dispersion) after aloe vera gel to avoid film wrinkling. The thickness of the films was measured by a micrometer and was equal to 150 ± 20 μm.

The composition of films with different combinations of polysaccharides was optimized by varying the mass ratio of carrageenan, starch, nanocellulose, and glycerol (indi-

cated as superscript). The developed compositions and their designations are presented in Table 1.

**Table 1.** Designations and composition of films based on carrageenan/starch.

Designation of Films	Mass Ratio of Carrageenan/Starch	Volume of Aloe Vera, mL	Volume of Nanocellulose, mL	Volume of Glycerol, mL
F1	1:2	2	2.5	-
F1-5	1:2	2	5	-
F2	1:1	2	2.5	-
F2-5	1:1	2	5	-
F3	1.5:1	2	2.5	-
F3-5	1.5:1	2	5	-
F3 <sup>gl</sup>	1.5:1	2	2.5	0.5
F4	2:1	2	2.5	-
F4-5	2:1	2	5	-

## 2.4. Investigation of Films

### 2.4.1. Fourier-Transform Infrared Spectroscopy (FTIR)

The structure of films was studied using an IRAffinity-1S spectrometer (Shimadzu, St. Petersburg, Russia) with an attenuated total reflectance accessory Quest Single Reflection ATR (Shimadzu, St. Petersburg, Russia) by the frustrated total internal reflection method at ambient temperature (without sample preparation, by single reflection) with 2 cm<sup>-1</sup> resolution and 45 scan numbers in the range of 400–4000 cm<sup>-1</sup>.

### 2.4.2. Scanning Electron Microscopy (SEM)

To investigate the inner and surface morphology, films were studied using a Zeiss Merlin SEM microscope (Carl Zeiss SMT, Oberkochen, Germany) at low 1 kV accelerating voltage and 100 pA electron beam current to prevent surface charge and modification during SEM [43]. The film cross-section was obtained by submerging of the film in liquid nitrogen for about one minute and fracturing perpendicular to the film surface in the liquid nitrogen after the Leidenfrost point was reached [44]. After this, the film sample was removed from liquid nitrogen and dried in air for 5 min.

The nanoscale cellulose structure was determined by the SEM method. The nanocellulose aqueous dispersion (~0.1 g) was dissolved in 50 mL water with the following ultrasound treatment. Then, a drop of this diluted solution was deposited onto a mica substrate and dried at 90 °C for 24 h. The solid residue on the substrate was further studied by SEM method, and the size of nanocellulose fibrils was calculated based on SEM micrographs.

### 2.4.3. Atomic Force Microscopy (AFM)

To evaluate surface topography, films were investigated using an NT-MDT NTegra Maximus atomic force microscope (NT-MDT Spectrum Instruments, Moscow, Russia) with standard silicon cantilevers (15 N/m rigidity) in the tapping mode [45].

### 2.4.4. Contact Angle Measurements

The sessile drop method [46] was applied to measure the water contact angle for films using a Goniometer LK-1 instrument (NPK Open Science Ltd., Krasnogorsk, Russia). The “DropShape” software (the Laboratory of Mathematical Methods of Image Processing, Lomonosov Moscow State University, Moscow, Russia) was applied to analyze the obtained data. The process was as follows: a drop was squeezed onto a needle, the edges of the drop were focused, the needle was lowered, and the drop sat on the surface of the film. Contact angles were measured at 3–4 s with frame intervals of 0.5 s and a water volume of 2 µL was used. At least seven different locations of the films were measured and the average contact angle values were presented.

#### 2.4.5. Mechanical Properties

Mechanical properties of films were investigated by the Shimadzu AG-50kNXD auto-graph (Shimadzu, Kyoto, Japan) using ASTM D638 [47], ISO 527-2 [48] protocols at a test speed of 100 mm/min.

#### 2.4.6. Thermogravimetric Analysis (TGA)

The thermochemical properties of films were determined using a TG 209 F1 Libra thermobalance (Netzsch, Leuna, Germany) with the speed of 10 K/min under an Ar atmosphere in the range of 30–530 °C. The sample mass was ~1.5–2 mg.

#### 2.4.7. Water Vapor Permeability (WVP)

The gravimetric method was used to measure WVP (g/(s × m × Pa)) for films using modified ASTM E96-95 [49,50]. The film was soldered to a weighing bottle containing silica gel to obtain a relative humidity (RH) of 0% under the film. This weighing bottle was left in a desiccator with a saturated sodium chloride solution to maintain a 75% RH. Water vapor passed through the film and absorbance by the desiccant was established by measuring the increase in weight using Equation (1):

$$WVP = \frac{\Delta m \times l}{t \times A \times P \times \Delta RH'} \quad (1)$$

where  $\Delta m$  is weight difference (g);  $l$  is the average thickness of film (m);  $t$  is experiment time (s);  $A$  is the permeation area (bottle neck area, m<sup>2</sup>);  $P$  is the partial water vapor pressure at 25 °C (3.169 kPa);  $\Delta RH$  is the difference in relative humidity (0.75).

#### 2.4.8. Oil Permeability (OP)

Oil film permeability was determined according to Wang et al. [51] as follows: a film with the size of 8 × 2 cm was placed onto a filter paper dried to a constant weight ( $w_i$ ) followed by uniform deposition of 25 oil drops ( $m_{oil}$ ) on the film surface for 24 h without leaving the edges of the film. Afterwards, the film with oil was removed, and the filter paper was weighed ( $w_f$ ). Oil permeability for the film was calculated using Equation (2):

$$OP = \frac{w_f - w_i}{m_{oil}} \times 100\%. \quad (2)$$

#### 2.4.9. Moisture Absorption (MA)

The determination of moisture absorption (MA) was carried out according to the method of Angles and Dufresne [52]. Films (2 × 2 cm) were dried at 60 °C until a constant weight ( $w_i$ ) and left in a desiccator with saturated sodium chloride solution to maintain a 75% RH. Samples were weighed after 24 h ( $w_f$ ). The MA was calculated using Equation (3):

$$MA = \frac{w_f - w_i}{w_f} \times 100\%. \quad (3)$$

#### 2.4.10. Solubility in Water

Solubility in water ( $Sw$ ) of films was measured according to Bierhalz et al. [53] as follows: film samples (2 × 2 cm) dried at 60 °C for 24 h to a constant weight ( $w_i$ ) were immersed in weighing bottles containing 15 mL of distilled water and left at 25 °C for 1 h. Afterwards, the films were dried in the same condition at 60 °C for 24 h and weighed ( $w_f$ ). The  $Sw$  was calculated using Equation (4):

$$Sw = \frac{w_i - w_f}{w_i} \times 100\%. \quad (4)$$

#### 2.4.11. Density Measurement

The determination of film density ( $\text{g}/\text{cm}^3$ ) was carried out using the flotation method at  $22\text{ }^\circ\text{C}$  [54]. A piece of film was placed in a graduated cylinder filled with a mixture of carbon tetrachloride and toluene (1:1 by volume). To take an equilibrium position using film in the middle of the liquid column, a low or high density liquid was added. Toluene ( $0.87\text{ g}/\text{cm}^3$ ) and carbon tetrachloride ( $1.48\text{ g}/\text{cm}^3$ ) were chosen due to demonstrating no reaction with the film. Then, the density of the liquid mixture was measured using a pycnometer. The film density was measured at least in triplicate.

#### 2.4.12. Film Light Transmission

The determination of film light transmission and transparency was carried out by spectrophotometry [50] using a Spectrophotometer PE-5400UV. The light barrier properties of the film sample ( $10\text{ mm} \times 45\text{ mm}$ ) were measured using a glass cuvette and air as a control at a wavelength between 200 and 800 nm.

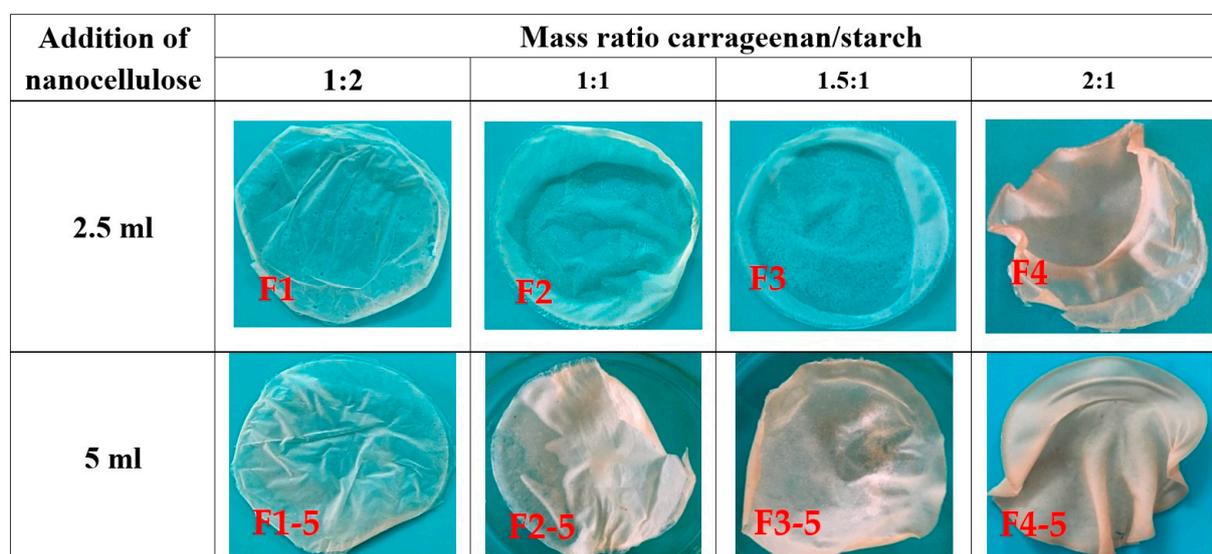
#### 2.4.13. Statistical Analysis

The data presentation was as mean  $\pm$  standard deviation (SD). At least three results of parallel measurements were obtained. The normal distribution of random variables was confirmed. Outliers were discarded from subsequent statistical analysis. Using formulas corresponding to the normalized normal distribution, the result of the analysis and its random error were calculated. Next, the total non-excluded systematic and general error of the analyses results were calculated and presented.

### 3. Results and Discussion

#### 3.1. Optimization of Film Composition

To choose the optimal composition, edible films were prepared with different combinations of polysaccharides (carrageenan, starch, and nanocellulose) (Figure 1).



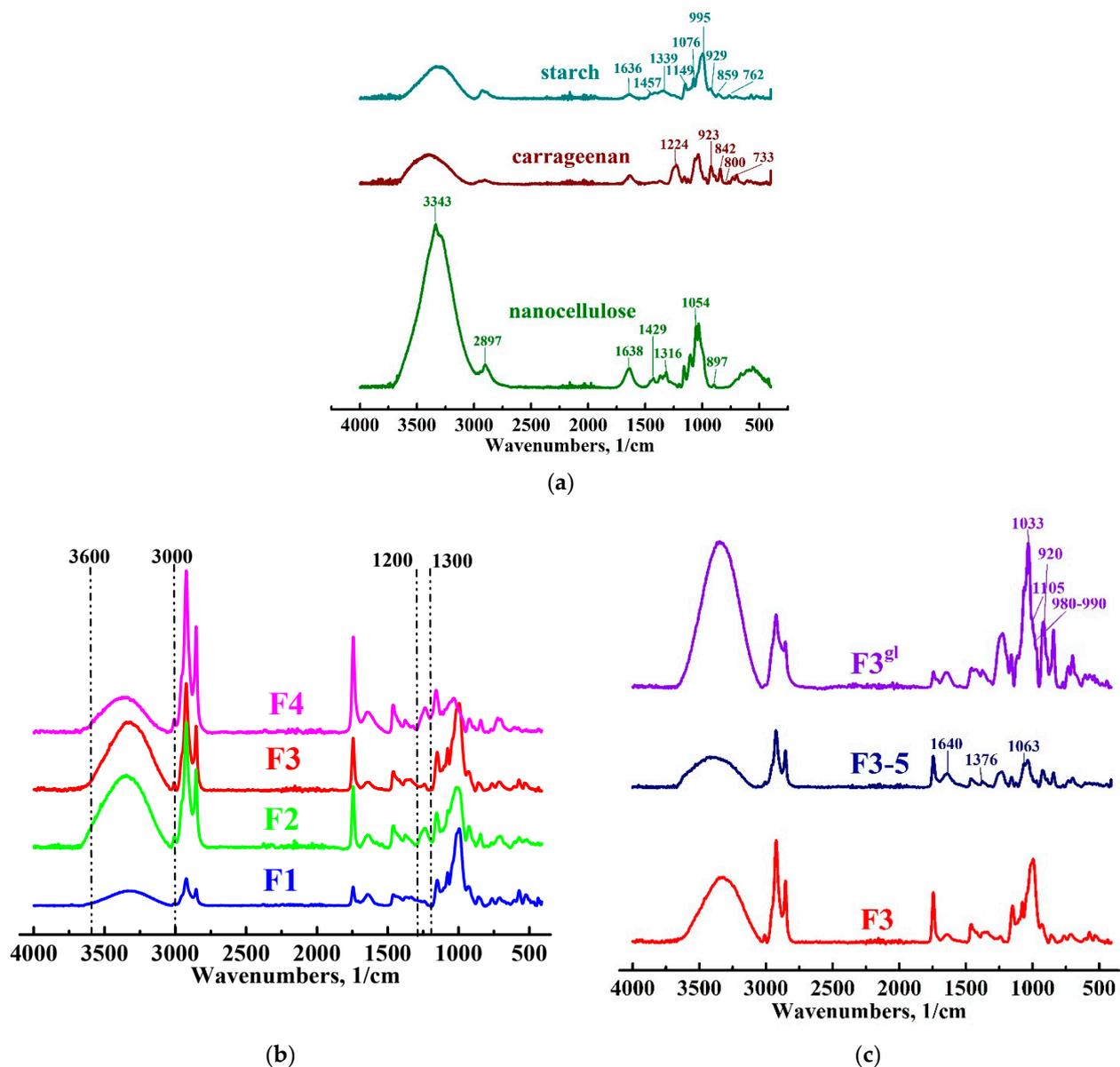
**Figure 1.** Photographs of films with different combinations of polysaccharides. To assess transparency and defects, the obtained samples were placed on a blue background.

The increase in the carrageenan and nanocellulose content in the film led to turbidity due to their color and, as a result, to a deterioration in the transparency of the films (Figure 1). This is unsatisfactory, since this will result in an opaque edible food packaging [50]. For industrial purposes, the films must have sufficient transparency [55]. Transparency is an important technical parameter for the evaluation and verification of certain food packaging materials [56]. Based on these data obtained, the F3 film with carrageenan/starch ratio of

1.5:1 and 2.5 mL nanocellulose addition was chosen as optimal. To avoid film wrinkling, glycerol was added to the F3 film composition (F3<sup>gl</sup> film).

### 3.2. Film Structure Investigation

The structure of polysaccharides (carrageenan, starch, nanocellulose) obtained and the films were investigated using FTIR spectroscopy, SEM, and AFM methods. FTIR spectroscopy (a non-destructive and rapid technique) was used to characterize the polysaccharides to confirm the substances extracted and the films in terms of possible molecular interactions and miscibility of polymer blend [57]. The FTIR spectra are presented in Figure 2.



**Figure 2.** FTIR spectra of (a) polysaccharides (carrageenan, starch, nanocellulose), (b) films with different carrageenan/starch ratios and 2.5 mL nanocellulose (F1, F2, F3, F4), and (c) films with carrageenan/starch ratio of 1.5:1 and different nanocellulose amount (F3, F3-5 and F3<sup>gl</sup>).

It was shown that the obtained FTIR spectra of carrageenan and starch (Figure 2a) corresponded to those presented in the literature. For all samples, a broad peak in the region of 3600–3000  $\text{cm}^{-1}$  related to the OH stretching vibration of the hydroxyl group of

polysaccharides and water absorption [57,58]. Peaks in the range 2900–2800  $\text{cm}^{-1}$  attributed to C-H stretching vibrations are responsible for the material lipophilicity [59]. The peaks in the range 700–1300  $\text{cm}^{-1}$  relating to the carbohydrate region are called the fingerprint region, and these bands are specific and allow the detection of each polysaccharide [57]. Characteristic peaks of carrageenan were observed (Figure 2a): at 1224  $\text{cm}^{-1}$  (ester sulfate groups), 923  $\text{cm}^{-1}$  (3,6-anhydrogalactose group), 842  $\text{cm}^{-1}$  (galactose-4-sulphate), 800  $\text{cm}^{-1}$  (3,6-anhydro-D-galactose-2-sulfate indicating impurities in sample), and 733  $\text{cm}^{-1}$  (3, 6-anhydro-D-galactose) [17,57]. The following characteristic peaks of starch were observed: at 1636  $\text{cm}^{-1}$  (C-O associated with the OH group or tightly bound water), 1457  $\text{cm}^{-1}$  (symmetric deformation of  $\text{CH}_2$ ), 1339  $\text{cm}^{-1}$  (carboxyl groups), 1149  $\text{cm}^{-1}$  (C-C and C-O stretching), 1076  $\text{cm}^{-1}$  (functional C-O groups), 995  $\text{cm}^{-1}$  (C-O stretching), and 929, 859, and 762  $\text{cm}^{-1}$  (ring vibration C-O-C of carbohydrate) [58,60].

The obtained nanocellulose FTIR spectrum (Figure 2a) corresponds to the previously published spectrum of nanocellulose [61]. The broad peaks at 3343  $\text{cm}^{-1}$  indicate the frequency of O-H stretching of both free and intermolecular hydrogen-bonded hydroxyl groups present in cellulose. The peak at 2897  $\text{cm}^{-1}$  relates to the C-H stretching frequencies of nanocellulose, 1638  $\text{cm}^{-1}$ —to the O-H vibrations of absorbed water, and 1429  $\text{cm}^{-1}$  refers to the planar (scissor) vibrations of the C-H bond of the methylene group of cellulose. The average peak around 1316  $\text{cm}^{-1}$  shows the bending vibration of the O-H bond of the alcohol group, and at 1054  $\text{cm}^{-1}$ —the stretching frequency of the C-O bonds of the C-O-C pyranose [62]. The peak around 897  $\text{cm}^{-1}$  is associated with  $\beta$ -glycosidic bonds [61,63].

For all film samples (Figure 2b), the broad peak ranging at 3600–3000  $\text{cm}^{-1}$  corresponded to OH stretching vibration from the hydroxyl group of polysaccharides and water absorption [57]. The increase in this peak also indicates higher water absorption [58]. It was demonstrated that a decrease in the content of one or another component (starch or carrageenan) led to a shift and an intensity decrease in its characteristic bands [57]. The shift of the bands in the region at 1200–1300  $\text{cm}^{-1}$  is due to the interactions between the charged functional groups of polysaccharides [17].

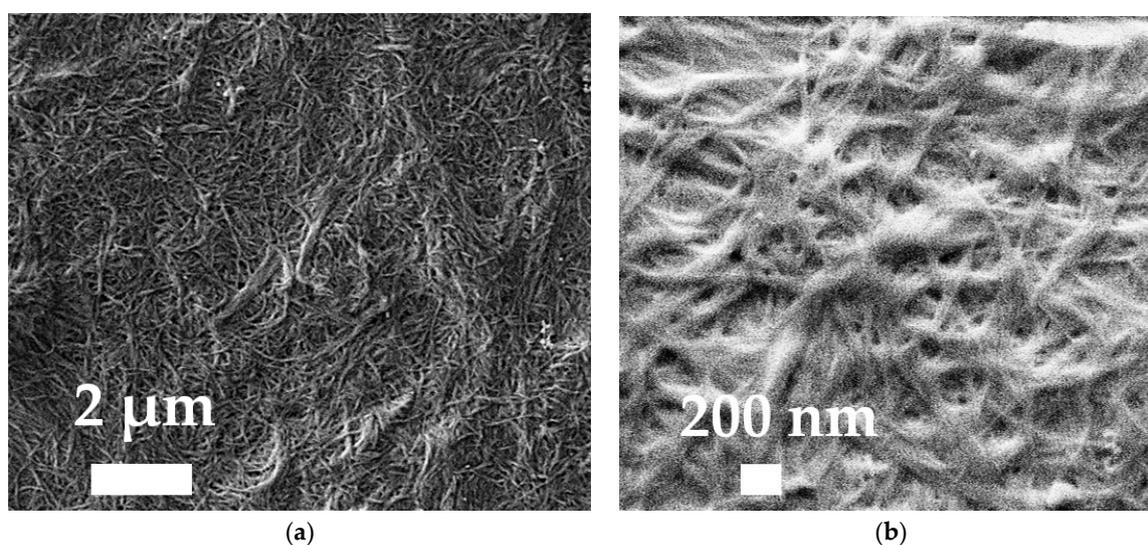
The increased nanocellulose content in the F3-5 film (Figure 2c) led to an intensity increase in its characteristic peaks compared to the F3 film: at 1640  $\text{cm}^{-1}$  related to the O-H vibration of absorbed water, 1376 and 1063  $\text{cm}^{-1}$  attributed to C-O and C-H groups in the polysaccharide rings, and C-O-C in the pyranose ring of cellulose, respectively [62,63]. The addition of glycerol into the F3 film led to the next changes in the FTIR spectrum (Figure 2c): the increased peak intensity at ~1105 and 1033  $\text{cm}^{-1}$ , the appearance of the shoulder in the range of 980–990  $\text{cm}^{-1}$  and the peak at 920  $\text{cm}^{-1}$ , which corresponded to the main characteristic peaks of glycerol [64]. These changes may indicate hydrogen bonding between polysaccharides and glycerol, and confirmed the plasticization of the F3<sup>gl</sup> film [65].

Based on SEM micrographs, the sizes of nanocellulose particles were calculated (Figure 3):  $20 \pm 10$  nm average diameter with a few hundred nanometers for average length. It should be also noted that the cellulose material mostly consists of nanofibrils.

The cross-section and surface morphology of the film was studied by SEM method. The cross-sectional SEM micrographs, surface SEM micrographs, and AFM images of films are presented in Figures 4 and 5, respectively.

The morphology structure of films is mainly affected by the ratio of starch and carrageenan in the initial dispersion. It was demonstrated that cross-sections of F1, F2, and F3 films had insoluble starch granules [66]; the number of intact ones increased with an increase in the concentration of starch in the films. Starch particles migrated to a greater extent on the upper film surface during its formation (confirmed also by surface SEM micrographs in Figure 4). Carrageenan acted as a binding matrix coating the starch particles that could lead to the improvement in film mechanical properties with the increase in its content (confirmed below in Section 3.3). The increase in nanocellulose content in the films did not significantly affect the cross-sectional structure. The introduction of glycerol into the F3 film (the F3<sup>gl</sup> film) led to the least amount of intact starch granules, which had larger

sizes impacted to less homogeneous than the initial dispersion [67], and caused a more compact structure without cracks or pores compared to the F3 film [65].



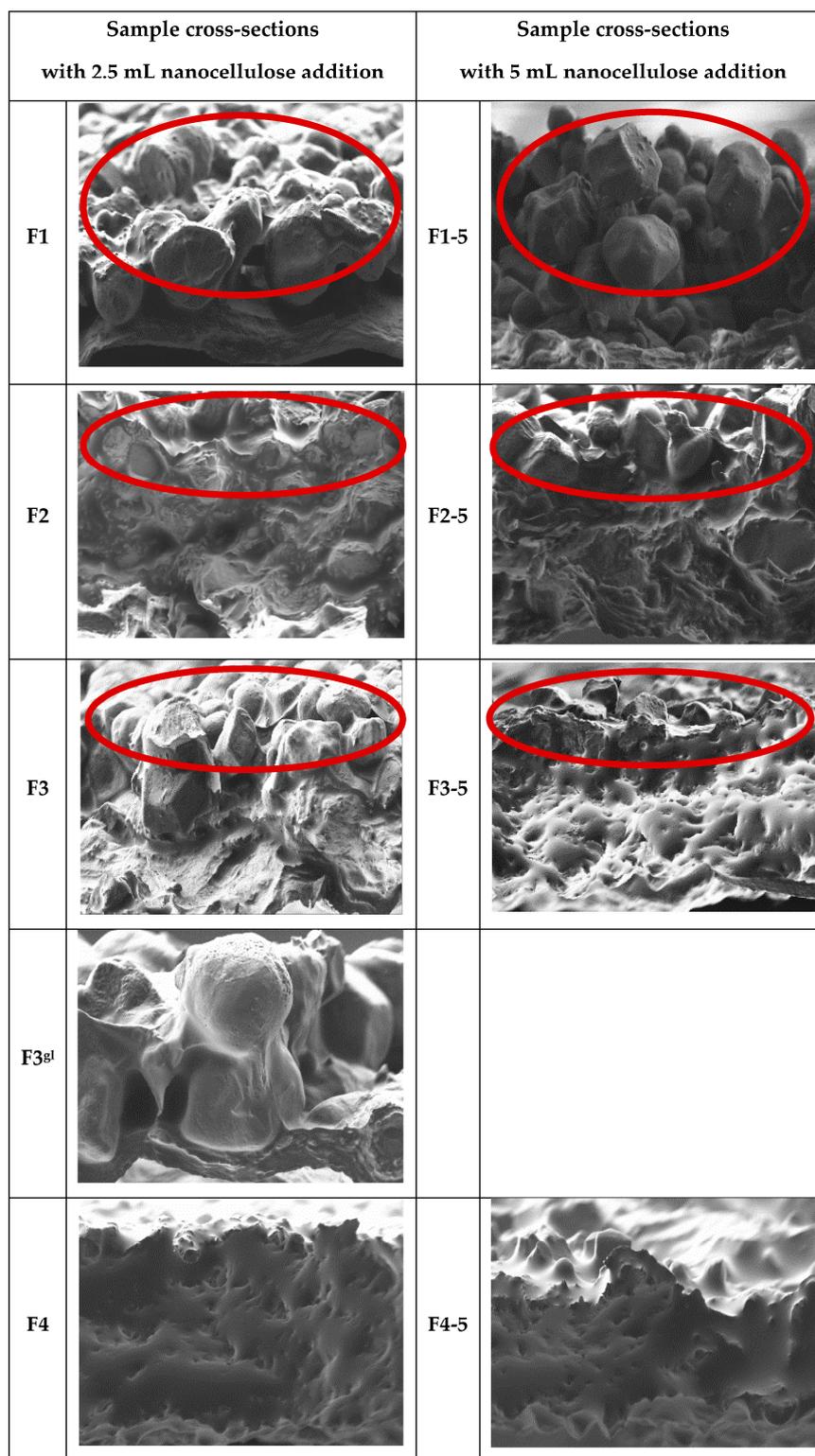
**Figure 3.** SEM micrographs of nanocellulose at different magnifications of (a) 8.15 KX and (b) 30.00 KX.

All films have harsh surfaces and starch granules on their surfaces, a number of which decreased with an increase in the content of carrageenan, which envelops them (Figure 5) [66]. The underneath surface structure for all films was much smoother than the top due to film preparation conditions (casting on glass Petri dishes). The F4 film with the highest carrageenan content was demonstrated to have the most uniform surface, but still with starch grains. It was demonstrated that a greater amount of carrageenan in the matrix led to better component dispersion. However, it did not seem advisable to choose this film due to its opacity (Figure 1). The AFM method is a powerful tool for obtaining not only qualitative but also quantitative information for studying the surface topography of various films [66]. Surface AFM images obtained for all samples were also in agreement with surface SEM data. Based on AFM data, surface roughness parameters in terms of the average (Ra) and root-mean-squared (Rq) roughness values were calculated (Table 2).

**Table 2.** Surface roughness parameters (Ra and Rq) of films.

Samples	Surface Roughness Parameters	
	Ra, nm	Rq, nm
F1	30.4 ± 5.0	43.7 ± 8.4
F1-5	27.2 ± 3.7	34.5 ± 5.6
F2	18.9 ± 2.6	25.7 ± 4.2
F2-5	19.1 ± 3.0	24.1 ± 3.9
F3	18.1 ± 2.5	23.7 ± 3.3
F3-5	16.6 ± 2.0	20.9 ± 2.9
F3 <sup>gl</sup>	12.3 ± 1.8	15.7 ± 2.4
F4	25.3 ± 3.5	35.7 ± 6.5
F4-5	24.9 ± 3.1	33.3 ± 6.0

It was demonstrated that film surface roughness values (Ra and Rq) decreased with a decrease in starch content (except for the F4 film). The roughness of the F4 film was conditioned by the bulges of starch granules not compactly located on the surface [66]. An increase in nanocellulose content (till 5 mL) slightly smoothed the surface of the films, but the values were within the margin of error. The glycerol-plasticized F3<sup>gl</sup> film showed a smoother surface (less surface roughness parameters) compared to the F3 film, due to a more compact structure [68].



**Figure 4.** Cross-sectional SEM micrographs of films at magnification of 2.5 KX. Designations F1–F4 refer to varying the carrageenan/starch ratio in the film composition, the introduction of 5 mL of cellulose is designated as –5, glycerol was indicated as superscript. Designations of films and their compositions are presented in Table 1. Borders highlighted in red mark the places of the starch granules location.

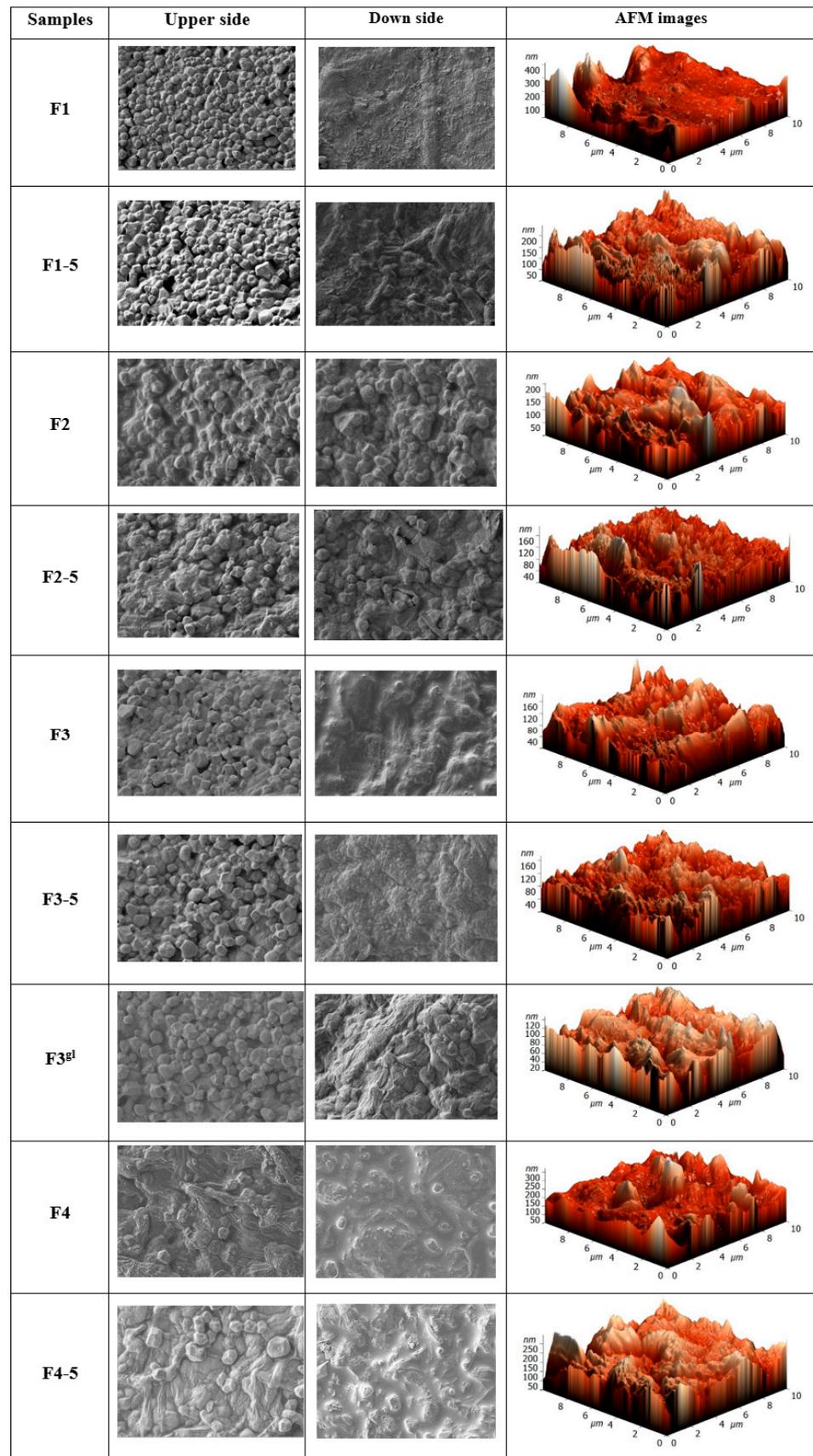


Figure 5. Surface SEM micrographs at magnification of 1 KX and AFM images of films.

### 3.3. Film Physicochemical Properties Investigation

Edible films must have satisfactory mechanical properties in order to be used as a packaging material at an industrial level [69]. Maximum tension and elongation parameters were measured for developed films (Table 3). To evaluate the hydrophilic–hydrophobic balance of film surface, contact angles of water were measured and presented in Table 3.

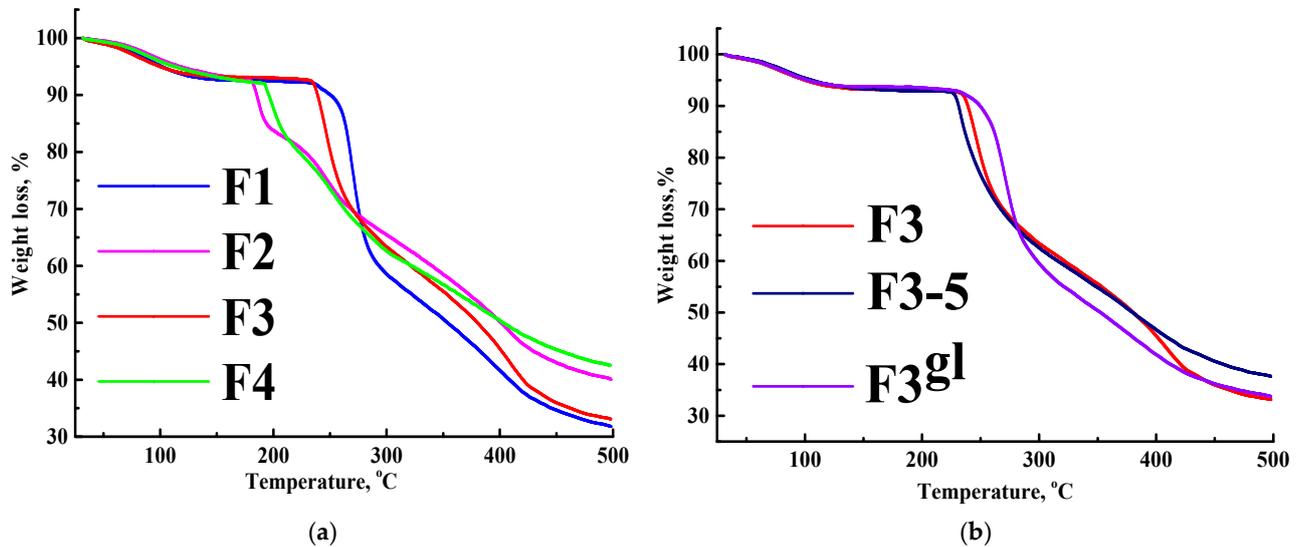
**Table 3.** Film mechanical properties in terms of maximum tension and elongation and water contact angles.

Samples	Mechanical Properties		Water Contact Angle, °
	Maximum Tension, MPa	Maximum Elongation, mm	
F1	-	-	38 ± 3
F1-5	0.65 ± 0.23	5.97 ± 2.20	35 ± 3
F2	0.08 ± 0.04	2.37 ± 1.19	63 ± 3
F2-5	1.54 ± 0.70	4.04 ± 1.85	58 ± 3
F3	0.59 ± 0.29	4.89 ± 2.41	74 ± 3
F3-5	2.70 ± 1.32	3.85 ± 1.85	73 ± 3
F3 <sup>gl</sup>	5.64 ± 2.34	4.62 ± 1.89	71 ± 3
F4	0.79 ± 0.35	5.15 ± 2.28	76 ± 3
F4-5	4.69 ± 1.91	2.72 ± 1.11	74 ± 3

It was demonstrated that a decrease in the content of starch in the film composition led to an improvement in the mechanical properties (maximum tension and elongation values) [70]; meanwhile, it was impossible to measure these parameters for the F1 film (carrageenan/starch ratio of 1:2) due to its fragility (Table 3). The introduction of nanocellulose used as the second reinforcing agent in the films offers improved mechanical stability in terms of maximum tension values [71] and provides impermeability towards moisture and gases [21]. However, the introduction of a larger amount of nanocellulose (5 mL) with a decrease in the starch content in the films (F1-5, F2-5, F3-5, F4-5) led to a decrease in the maximum elongation, which could be due to plastic deformations formed between polymers and nanocellulose (confirmed by SEM data in Figure 5) [72]. Under comparison of maximum elongation data for films containing 2.5 and 5 mL nanocellulose, it could be noticed that for films with a high content of starch (F1 and F1-5, F2 and F2-5) the increase in nanocellulose content led to the rise in the maximum elongation. For films with increased carrageenan content (F3 and F3-5, F4 and F4-5), the maximum elongation decreased with the rise in the nanocellulose concentration in the films. It could be due to the fact that starch had a greater effect than nanocellulose, because it was dissolved at room temperature and was a “reinforcing agent” more than a polymer film matrix. It is also worth noting that the introduction of glycerol (0.5 mL) into the F3 film not only prevented the film shrinking, but led to an improvement in its mechanical properties and flexibility (5.642 MPa and 4.620 mm for the F3<sup>gl</sup> film) [73]. Glycerol may reduce the intramolecular affinity between the polysaccharide chains by hydrogen bonding with them. As a result, the film matrix becomes less dense with easier movement of polymer chains, which caused greater film flexibility [65].

The water contact angle values for films with the same 2.5 mL nanocellulose, aloe vera gel, and oil content increased till 76° with the decrease in starch content in the film composition (Table 3), indicating the surface hydrophobization. This effect is possible, since carrageenan is less hydrophilic compared to starch. The value of water contact angle for the F4 film mainly from carrageenan was close to those obtained earlier [74]. The nanocellulose introduction of 5 mL slightly decreased the water contact angles because of the hydrophilic nature of nanocellulose [75,76]. The F3<sup>gl</sup> film has the lowest contact angle of water (71°) compared to the F3 and F3-5 films due to the introduction of glycerol. Earlier, it was confirmed that the higher glycerol content led to a lower contact angle of water [65].

During transportation and storage of products, the temperature may be varied. Thus, it was necessary to study the film stability at elevated temperatures by TGA. Thermogravimetric (TG) curves are shown in Figure 6.



**Figure 6.** TG curves for films with (a) with different ratios of carrageenan/starch and 2.5 mL nanocellulose (F1, F2, F3, F4), and (b) with carrageenan/starch ratio of 1.5:1 and different amount of nanocellulose addition (F3, F3-5, and F3<sup>gl</sup>).

There are three stages of weight loss for F1 and F3 films: (1) 7% at ~224 °C related to moisture loss and evaporation of the trapped water solvent, (2) 35% at 305 °C and 31% at 315 °C for F1 and F3 films, respectively, corresponding to decomposition of functional groups of film components, and (3) 26% at 494 °C and 28% at 498 °C for F1 and F3 films, respectively, determining the degradation of the sample matrix [77,78]. F2 and F4 films have only two stages of thermal decomposition: (1) 7% weight loss at 176 and 185 °C, and (2) 52% at 495 °C and 49% at 490 °C for F2 and F4 films, respectively. The decrease in the concentration of starch in films led to a lower weight loss and decomposition at a lower temperature (Figure 6a) [79]. To evaluate the nanocellulose effect on thermochemical properties, films with a carrageenan/starch ratio of 1.5:1 and a different amount of nanocellulose addition (F3, F3-5, and F3<sup>gl</sup> films) were also investigated (Figure 6b). It was demonstrated that an increase in nanocellulose content from 2.5 to 5 mL did not have a significant effect: the sample decomposition temperature increased from 315 to 327 °C, and the weight loss decreased from 68 to 60% [70,80]. The addition of the plasticizer-glycerol into F3 (the F3<sup>gl</sup> film) also does not affect the thermochemical properties of the film. Thus, the thermal analysis showed that developed edible films were stable up to 200 °C.

Based on these data obtained, the F3<sup>gl</sup> film with a carrageenan/starch ratio of 1.5:1, 2.5 mL nanocellulose, and 0.5 mL glycerol addition was chosen as the optimal product. For this film, the most important parameters were measured, such as thickness, density, water vapor (WVP) and oil (OP) permeability, water solubility (Sw), and moisture absorption (MA) (Table 4), which are necessary to evaluate the prospects of using an edible film as a packaging material.

**Table 4.** Thickness, density, water vapor (WVP) and oil (OP) permeability, water solubility (WS), and moisture absorption (MA) for films with carrageenan/starch ratio of 1.5:1.

Samples	Thickness, $\mu\text{m}$	Density, $\text{g}/\text{cm}^3$	WVP, $\text{g}/(\text{s} \times \text{m} \times \text{Pa})$	OP, %	Sw, %	MA, %
F3 <sup>gl</sup>	150 ± 10	1.34 ± 0.02	6.4 × 10 <sup>-10</sup>	2.0 ± 0.5	42 ± 10	29 ± 4

To prolong the shelf life of the products, the interaction between the packaged product and the environment must be kept to a minimum [81]. WVP is an important criterion for food packaging material to evaluate it. So low WVP value ( $6.4 \times 10^{-10}$  g/(s  $\times$  m  $\times$  Pa)) may be explained by the fact that water molecules can be easily absorbed and trapped in the film due to the reduced free volume between polymer chains and the film's hydrophilic nature (confirmed by contact angle data in Table 3). The F3<sup>gl</sup> film also had very low oil permeability value (2%) (practically impermeable to oil) because of the hydrophilic nature of the components, which makes it suitable for packaging fatty foods [50,51]. However, water solubility ( $S_w$ ) of this film was high due to two main water-soluble components, carrageenan and starch [81]. Therefore, the F3<sup>gl</sup> film is suitable as a packaging material for food with low water activity (for example, dried fruits and vegetables), but not suitable for ones with high moisture and wet surface (for example, meat and seafood) [50]. It should also be noted that the developed film can be used in humid environments (for example, in hot humid climates) as it showed little moisture absorption (MA of 29%). The light transmission of the F3<sup>gl</sup> film was evaluated (Figure 7).

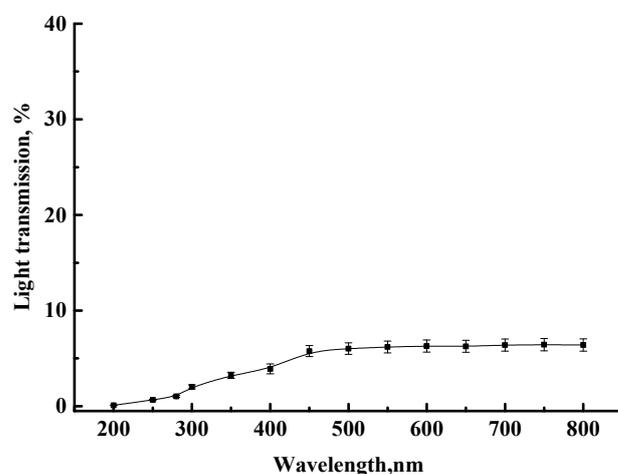


Figure 7. Light transmission properties of the F3<sup>gl</sup> film.

It was shown that the F3<sup>gl</sup> film had weak light transmission in the ultraviolet light region (till 400 nm). With an increase in the wavelength in the visible range of 400–800 nm, the light transmission of the film increased. Such a low light transmission of the film in the ultraviolet and visible range (Figure 7) can be useful in food packaging to slow down the spoilage of products.

#### 4. Conclusions

For the prospective creation of bio-based edible food packaging materials, edible and environmentally friendly antibacterial edible films were developed from carrageenan reinforced with starch and nanocellulose. The effect of the carrageenan/starch ratio variation and nanocellulose content on film structure and physicochemical properties was studied. The interactions between the charged functional groups of polysaccharides were confirmed by FTIR spectroscopy. The morphology investigated by SEM and AFM methods demonstrated the migration of starch particles on the upper film surface resulting in increased surface roughness, while carrageenan acted as a binding matrix coating the starch particles and causing improvement in the film's mechanical properties. Nanocellulose incorporated into the system also improved the film's mechanical properties. The increase in the carrageenan and nanocellulose content in the film led to turbidity due to their color and, as a result, to a deterioration in the transparency of the films. Thus, the film with a carrageenan/starch ratio of 1.5:1 and the addition of 2.5 mL nanocellulose was chosen as the optimal one, into which 0.5 mL glycerol was incorporated as a plasticizer hydrogen bonding with polysaccharides (confirmed by FTIR data). It led to a more compact

film structure without cracks or pores (confirmed by SEM data), a more hydrophilic and smoother film surface (confirmed by SEM, AFM, and contact angle data), and improved flexibility (confirmed by measurements of mechanical properties) and moisture resistance. The thermal analysis showed that all developed edible films were stable up to 200 °C (confirmed by TGA). The film with a carrageenan/starch ratio of 1.5:1 and addition of 2.5 mL nanocellulose and 0.5 mL glycerol had satisfactory properties in terms of water vapor permeability, oil permeability, water solubility, and moisture absorption. Based on these findings, it can be concluded that this developed film might have great potential to be used as a biodegradable edible food packaging material to avoid the use of plastic.

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