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Research article

Contribution of flax-cellulose nanocrystals on the structural properties and performance of starch-based biocomposite films

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Abstract. Thermoplastic starch (TPS)/cellulose nanocrystal (CNC) films with glycerol or sorbitol plasticizers at 30 and 40% concentrations were prepared by casting over the whole composition range. The nanocrystalline cellulose was extracted from bleached flax by sulphuric acid hydrolysis. The haziness, tensile strength, thermal stability, and the effect of storage on crystal structure were investigated. Water vapor sorption of the neat TPS and flax-CNC films and water vapor permeability of the composites were measured at various humidity differences. The results showed that the high aggregation tendency of flax-CNCs significantly increases the opacity of films regardless of the type and amount of plasticizer. The reinforcing effect of nanocrystals can only be exploited at small concentrations (0–5%). However, 3–4% flax-CNCs result in a 15–25% reduction in water vapor permeability. Retrogradation of the TPS also reduced without changing the thermal stability. The properties of composites and their compositional dependence differ significantly from those of the composites prepared previously with cotton-CNC. Thus, knowledge of the properties of CNCs is essential for explaining the properties of polymer/CNC composites and the compositional dependence of properties.

Keywords: *biopolymers, biocomposites, nanomaterials, polymer membranes, starch-based films, cellulose nanocrystals (CNC)*

1. Introduction

To protect the environment, replacing conventional – petroleum-based – plastics with biodegradable materials is advisable in many areas. Biodegradation of polymers means that they break down into low molecular weight compounds under mild conditions over a particular time, and the resulting products are not harmful to the environment. These properties are particularly advantageous for plastics used for short periods, such as packaging. Large quantities of renewable polymers, such as starch and cellulose, are present. These are cheap and biodegradable materials and have many other beneficial properties [1]. Thus, academic settings and the industry are increasingly focusing on natural-based polymers, including starch and cellulose. Their properties, however, are still lagging behind those of synthetic plastics, and their development is the aim of biopolymers research today.

Starch is a biopolymer that can easily be produced from various plant sources. Starch is composed of amylose and amylopectin macromolecules; their ratio depends on the starch source. The amylopectin content is generally higher, around 70–80%. Both polymers are made up of α-D-glucose units. Amylose

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is a linear polymer in which the monomeric glucose units are linked by α-D-(1→4) bonds. On the other hand, amylopectin has a branched structure, where α-D-(1→4) bonds are joined to the glucose units by α-D-(1→6) bonds. Branching points appear every 20–25 glucose units. The so-called cluster model most often describes its structure [2].

A plasticizer and special conditions (thermal treatment with moisture) are usually used to disrupt the stable structure of starch held together by hydrogen bonds during processing. The resulting material is called thermoplastic starch (TPS) [3]. Films made from TPS are transparent, have good oxygen and carbon dioxide barrier properties, are even edible but highly water-sensitive, and have poor tensile strength. In addition, during storage, their properties change, and physical aging or recrystallization may occur. The disadvantageous properties of TPS can be moderated by other polymers, fillers, fibers, cross-linking, and chemical modification. The possibilities are almost endless [4]. Biodegradability is of paramount importance today for protecting our environment. It may be why much research is devoted to modifying TPS with biopolymers. Such biopolymers are *e.g.*, agar, chitosan, pectin, and cellulose [5–8]. Experiments have been carried out with all three types of nanocelluloses, such as cellulose nanocrystal (CNC), cellulose nanofiber (CNF), and bacterial nanocellulose.

Cellulose nanocrystals are most easily obtained by acid hydrolysis of the amorphous regions of cellulose. The hydrolysis results in rigid needle crystals with a high specific surface area and a high aspect ratio. They have been the basis of much new research in the last decade. Even in small quantities, CNC can be used to produce products with unique properties and high added value [9, 10].

The effect of cellulose nanocrystals on the properties of TPS films has been widely investigated. In general, the CNCs improve the mechanical properties of starch and reduce its water sensitivity. 3% CNC was added to TPS, plasticized with glycerol, and made from corn starch to reduce the water sensitivity of films [11, 12]. The CNC was obtained from sugar cane. The results showed that the water vapor permeability of the TPS films was decreased significantly by CNC addition because the CNC needle-like crystals increased the length of the path required for water molecules to pass through the films in diffusion-controlled water vapor permeability. In the case of TPS-CNC films derived from potato starch and cotton cellulose, the water vapor transmission decreased with increasing CNC concentration, and the maximum tensile strength values were measured at 3% CNC content [13].

A comparative study was carried out with CNF and CNC from unknown cellulose sources to improve the properties of pumpkin starch (PS)-based films [14]. The CNC was added at 1 and 2%; the amount of CNF was 10 and 15%, based on the dry weight of starch. Results revealed that the PS/CNF films showed better thermal stability than PS/CNC films. The PS/CNC films, however, had a higher tensile strength. Both cellulosic nanomaterials decreased the transparency of the starch films. CNCs from unknown cellulose sources and unknown extraction methods were used as fillers for different starch samples derived from potato, corn, and pea [15]. Results revealed that the CNCs increased the rigidity, thermal stability, and moisture resistance of the TPS films.

Published results generally show that the effect of CNCs is positive and that cellulose nanocrystals contribute significantly to improving the properties of TPS films. However, despite the numerous results, many questions remain unclear as the results are often contradictory. In addition, the interactions are usually not quantitatively evaluated because of (1) the narrow concentration range of CNCs investigated and the fact that (2) the type and concentration of the plasticizer are not varied in the research. Furthermore, many publications do not specify the cellulose source and extraction method used for producing CNCs, although these substantially impact the properties of CNCs. Accordingly, the size and shape of the nanofillers, the aspect ratio, the surface charge, the aggregation tendency, *etc.*, can vary significantly. All of these significantly affect the impact and effectiveness of CNCs as nanofillers in shaping, for example, the properties of thin films.

Our previous comparative studies have demonstrated the difference between CNCs obtained from two of the most important and widely used cellulosic fibrous materials: bleached cotton and flax [16, 17]. Controlled sulphuric acid hydrolysis extracted the CNCs, and they differed significantly in size, morphology, stability, and transparency of their aqueous suspensions, as well as in the aggregation tendency of nanocrystals. For this reason, their effect in the TPS matrix may also be different. To demonstrate this, in the present study, we used nanocrystalline cellulose extracted from bleached flax by sulphuric acid hydrolysis to improve the properties of TPS films and compared the effect of flax-CNC with our previous results obtained with CNCs extracted from bleached cotton (cotton-CNC) in TPS matrix [18]. For plasticizing starch, glycerol or sorbitol was used at a 30 or 40% concentration. The flax-CNCs were added to TPS in a wide concentration range. Various measurements evaluated the optical, tensile, and barrier properties of the biocomposite films. Morphology and aging were also characterized. Additionally, we assessed the interactions between the TPS matrix, the CNC filler, and the plasticizer quantitatively.

2. Experimental

2.1. Materials

The nanocrystalline cellulose was prepared by sulphuric acid hydrolysis. Bleached linen plain-weave fabric provided by Pannon-Flax Linen Weaving Co., (Győr, Hungary) was used as the cellulose source. The fabric had an area density of approximately 165 g/m^2. The CNC was prepared using 64% sulphuric acid, diluted from 98% analytical grade sulphuric acid (Sigma-Aldrich). Native, edible corn starch produced by Hungrana Ltd. (Szabadegyháza, Hungary) was used for the experiments. Two types of plasticizers: 99.5% glycerol and sorbitol, both purchased from Sigma-Aldrich, were used for the research.

2.2. Sample preparation

The nanocrystalline cellulose was prepared from 10 g of bleached flax cellulose, which was first ground to powder in a Retsch MM400 ball mill (Retsch GmbH, Haan, Germany). The hydrolysis was carried out with 64% sulphuric acid treatment at 45 °C for 25 min. Deionized water was then added to the mixture to stop the reaction and allowed to settle in the refrigerator. The following day, the CNC suspension was washed several times, centrifuging at 13 500 rpm at 5 °C for 10 min between washing steps on a Hermle Z326K type refrigerated centrifuge (Labortechnik GmbH, Wehingen, Germany). The resulting suspension was dialyzed against tap water for five days, which proved sufficient time to neutralize the suspension. Dialysis was followed by ultrasonication performed at 60% amplitude for 10 min in an ice bath using a Sonics VCX-500 (Sonics & Materials, Inc., Newtown, CT, USA) ultrasonic immersion transducer. The suspension was then stored in a refrigerator.

The final aqueous suspensions contained about 3% cellulose nanocrystals by weight. The average yield was about 34%, calculated as a percentage of the initial weight of flax powder. The average length and width of CNC whiskers obtained from flax-cellulose were 57 and 6 nm, respectively [16].

A 3% starch solution, freshly prepared each time before film casting, was used for the experiments. The preparation of the TPS solution was as follows: native starch (6 g) was dispersed in 200 ml of distilled water containing 30 or 40% (by weight of starch) of a plasticizer (*e.g.*, glycerol or sorbitol). The suspension was continuously stirred with a magnetic stirrer (Heidolph MP 3001, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 80 °C for 30 min at 400 rpm to gelatinize the corn starch particles. The solution was allowed to stand for 1 hour and then ultrasonicated at 60% amplitude for 1 min.

Before casting the TPS-CNC films, the CNC suspension was added in different percentages (0,1, 2, 3, 4, 5, and from 20 to 100% in 20% steps, on the starch basis) to the cold aqueous gelatinized starch. The mixture was ultrasonicated for 30 s, as described above. Rectangular films with a thickness of about 70 µm were cast from the TPS-CNC suspensions on the surface of a polypropylene plastic sheet. It was observed that after evaporating at room temperature for about 2 days, the films were released from the plastic sheet. And then, they were easily detachable. The detached films were stored and tested in a conditioning room at 23 °C and 50% relative humidity (*RH*). Storage time was 1 week, 2 months, and 5 months. In some experiments, only selected compositions of TPS-CNC films were investigated because of the wide composition range studied in the research.

2.3. Characterization

All the films were transparent. To illustrate the transparency, photographs were taken of the films. In addition, the haze index was determined in triplicate to characterize the transparency. A HunterLab ColorQUEST XE spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, USA) was used in full transmission mode.

Mechanical properties were determined using an Instron 5566 tensile tester (Instron Corporation, Norwood, MA, USA) equipped with a 500 N load cell. At least ten specimens with a size of 7×50 mm were cut from each of the films in different series. They were tested at a 10 mm/min crosshead speed and with a 20 mm span length.

Water vapor sorption (*WVS*) measurement was only performed on 100% starch (without CNC) and 100% CNC films with 30% sorbitol and 30% glycerol as a plasticizer. Three parallel measurements of each were performed. The samples were placed in Petri dishes and then kept in a Memmert-type air-circulation climate chamber (Memmert GmbH, Schwabach, Germany) with an *RH* of 80% at 25 °C. The mass of the samples was weighed at fixed intervals until the equilibrium water uptake was reached.

Water vapor permeability (WVP) was investigated only for TPS-CNC films containing 30% glycerol plasticizer at 0, 1, 3, 5, and 100% CNC content. The film under test was sealed in the mouth of the jar containing distilled water that provides the 100% *RH* condition. The test jar was kept in a Memmert-type air-circulation climate chamber with an RH of 30, 50, and 70% at 25 °C. The sealed jar was periodically removed and weighted. The decrease in the weight of water in the jar was used to calculate the WVP of the sample. The Equation (1) was used for calculation:

$$WVP = \frac{\Delta m}{\Delta t}\frac{x}{A\Delta p} \tag{1}$$

where x is the thickness of the film, A is the test area, $\Delta m/\Delta t$ is the slope of the straight line (weight loss per unit time), and Δp is the difference between water vapor partial pressure inside and outside the cup.

Thermal testing was carried out on films containing 30% sorbitol and 30% glycerol plasticizer at the following starch-CNC compositions: 100–0, 99–1, 98–2, 97–3, 96–4, 95–5, 90–10, 80–20, 50–50, 0–100%. Thermal decomposition of the films was studied by thermogravimetric analysis (TGA) using a Perkin Elmer TGA6 apparatus (PerkinElmer Inc., Waltham, Massachusetts, USA) at a heating rate of 10 °C/min, under N_2 atmosphere from 30 to 700 °C.

X-ray diffraction (XRD) on films containing 30% glycerol plasticizer was performed on the following TPS-CNC compositions: 100–0, 97–3, 0-100%. The films were stored in Petri dishes at 23 °C in a 50% *RH* atmosphere for several months. The same films were tested three times during storage, after 1 week, 2 months, and 5 months to determine the effect of storage on the crystal structure. Measurements were performed at the 4–40° with 0.04° step intervals and 1 s sample collection time on a Philips PW PW1830/PW1050 diffractometer (Koninklijke Philips N.V., Amsterdam, Netherlands) with Cu K_α radiation at 40 kV and 35 mA.

3. Results and discussion

3.1. Structure

Thin, easy-to-handle, transparent, and colorless films were prepared by casting and evaporation from the TPS and flax-CNC suspensions in a wide composition range. First, the films were visually inspected to compare their appearance and transparency. Figure 1 shows photographs of films made with different types and amounts of plasticizer without CNC. The comparison suggests that the TPS films with higher plasticizer content have better transparency, presumably because these films have better homogeneity. The film with the best transparency is the one containing 40% sorbitol. Similar results were obtained in our previous studies [18].

The relationship between the appearance and the structure of the films can also be well investigated by measuring the haze index, which gives the haziness of the samples and is inversely proportional to the transmittance. Figure 2 shows the haze index of TPS/CNC nanocomposites prepared with different types and amounts of plasticizer as a function of CNC content. It is evident that as the amount of nanocellulose particles increases, the haziness of the films increases. With increasing CNC content, the aggregation tendency of nanoparticles increases, and the large aggregates scatter light better. However, no significant difference in the haze index can be seen depending on whether glycerol (Figure 2a) or sorbitol (Figure 2b) was used as a plasticizer. Indeed, samples with a smaller plasticizer content scatter light slightly better in the case of sorbitol, but the situation is not so clear for glycerol.

It is worth comparing the haziness of TPS nanocomposites containing CNCs made from flax and cotton. Our previous studies have shown that CNCs made from two different materials have different tendencies to aggregate. In the flax-CNC suspension, the laser diffraction particle size analysis showed the presence of 10.9 μm aggregates. They were formed by nano-sized needle crystals, as the TEM records proved. However, in the cotton-CNC suspension, the nanocrystals only formed aggregates with an average size of 2.5 μm [16].

Since the starting properties of TPS are significantly affected by the manufacturing and storage parameters, it is preferable to compare the changes in the

Figure 1. Transparency of thin flax-TPS films plasticized with glycerol (c) and (d) or sorbitol (a) and (b) at two different concentrations: 30% (a) and (c) and 40% (b) and (d). (The text on the paper used as background is not relevant.)

haze index due to CNC dosing rather than comparing the absolute haze indices. Figures 2c and 2d show the change in the haze index as a function of CNC content. For this comparison, the cotton-CNC data are calculated from the results published previously elsewhere [18]. It is obvious that the flax-based nanocellulose results in a much more significant change in the haze index of the TPS samples than the cotton-based CNC, regardless of the type and amount of plasticizer. This statement is entirely consistent with our previous results showing that flax-CNC aggregation is much more significant than cotton-CNC [16]. It is interesting and difficult to explain how the opacity-enhancing effect of flax-CNC depends on the type and amount of plasticizer. At 30% plasticizer, the opacity of samples made with glycerol and at 40% with sorbitol increases more with CNC content.

3.2. Mechanical properties

It is well known that if a filler has a reinforcing effect, it increases the strength of the polymer in addition to its modulus [19]. Figure 3 show the tensile strength of TPS nanocomposites as a function of composition. It is obvious that the strength-enhancing effect of CNC can only be observed at very low concentrations (Figure 3a); however, the curves tend to saturate with higher CNC concentrations (Figure 3b). In contrast, in the case of cotton nanocelluloses, a strength increase was observed practically over the entire composition range investigated (0–60% CNC) [18]. As already mentioned in the discussion of the haze index, flax-CNC is more prone to aggregation than cotton-CNC. So even at very low CNC contents, the aggregation can be so significant that the strength-enhancing effect of the individual particles is no longer noticeable.

Previous research has demonstrated (1) nanocellulose size and morphology, (2) processing methods, and (3) matrix-filler interactions have a significant impact on the mechanical properties of nanocomposites [20, 21]. In addition to these factors, present results demonstrate that the aggregation tendency of

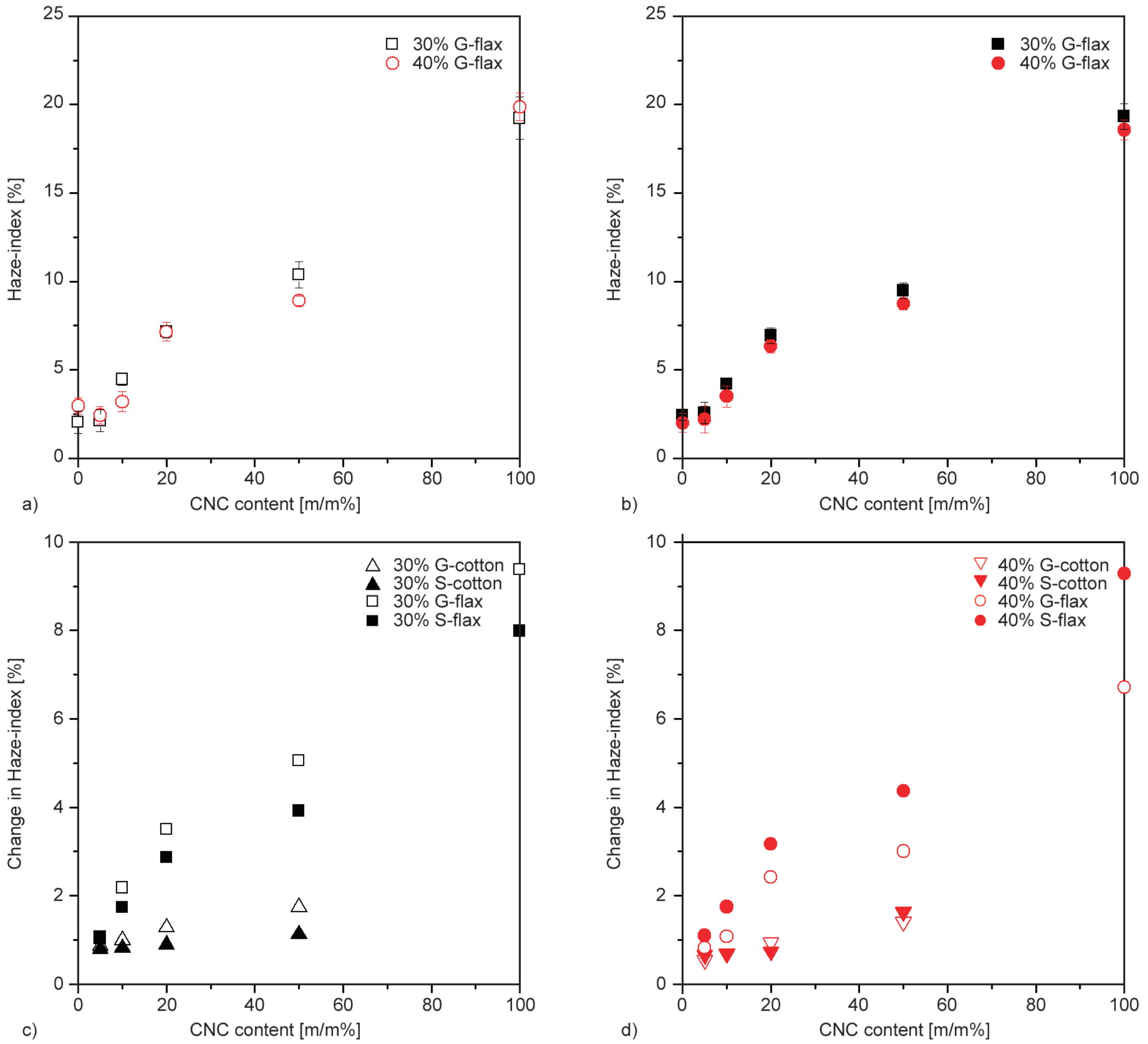

Figure 2. The effect of flax-CNC content on the haze-index of TPS nanocomposites prepared with different plasticizers a) glycerol (G), b) sorbitol (S); Change in haze-index of TPS-CNC films as a function of CNC content: the effect of type (G: glycerol; S: sorbitol) and concentration 30%, c) and 40%, d) of the plasticizers and the source of cellulose (flax, cotton) on the change in haze-index of TPS films occurred by CNC addition. Data of TPS-cotton-CNC films c) and d) are calculated from the results published previously elsewhere [18].

nanocrystalline cellulose is also a key factor in the development of the mechanical properties of the composite. In turn, the aggregation propensity may be influenced by the cellulose source [16].

The type and amount of plasticizer significantly affect the mechanical properties of TPS [22]. Increasing the plasticizer content decreases the stiffness and strength and increases the deformability. Sorbitol is a poorer plasticizer of starch than glycerol, so the same amount of sorbitol causes a smaller decrease in modulus and strength. These effects can be clearly observed in Figure 3. The TPS-nanocomposites with 30% sorbitol content have the highest strength, and those with 40% glycerol content have the lowest strength. From Figure 3, however, it can also be assumed that the type and amount of plasticizer only determine the properties of the matrix polymer and has less influence on the effect of the CNC. It is indicated by the fact that the slope of the curves describing the composition-dependence of tensile strength depends little on the type and amount of plasticizer. We can verify this hypothesis by examining the reinforcing effect of CNC by using an appropriate model.

3.3. Reinforcement of CNC in TPS nanocomposites

As was previously shown, the type and amount of plasticizer can be a significant effect on the mechanical properties. Plasticizers can influence all interactions (in the TPS matrix and aggregates or between

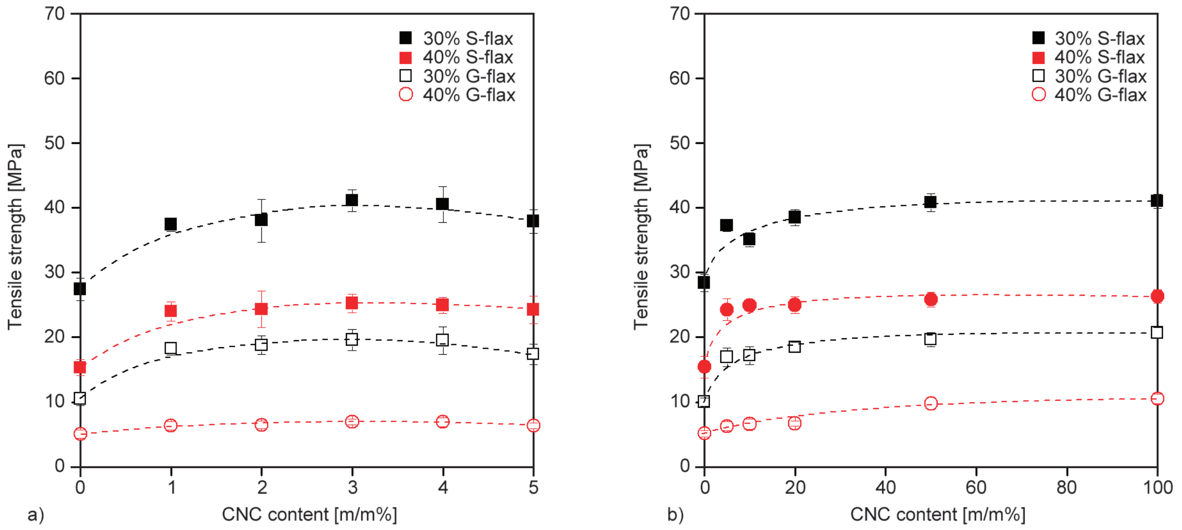

Figure 3. Tensile strength of TPS-flax-CNC films plasticized with glycerol (G) or sorbitol (S) at narrow (a) and wide (b) concentration ranges of CNC (Lines are drawn only to guide the eye, and they are not fitted correlations).

starch and CNC). Interactions can be analyzed quantitatively from the composition dependence of tensile strength by using a simple model (Equation (2)) [23]:

$$\sigma_{\mathrm{Tred}} = \sigma_{\mathrm{T}}\frac{1+2.5\varphi}{1-\varphi}\frac{1}{\lambda^{\mathrm{n}}} = \sigma_{\mathrm{Tm}}\exp(B\varphi) \tag{2}$$

where σ_{T} and σ_{Tm} are the true tensile strength of the TPS–CNC composite and the neat TPS, respectively, $\sigma_{\mathrm{T}} = \sigma_{\lambda}$ and $\lambda = L/L_0$, where L is the ultimate and L_0 the initial gauge length of the specimen, n is a parameter taking into account strain hardening, φ is the volume fraction of CNCs that was estimated from the weight fraction by using the density of the components, σ_{Tred} is the reduced tensile strength, and B is the relative load-bearing capacity of TPS and CNCs. Parameter B is the component of the model that can be related to interactions. The value of B can be easily determined by taking the logarithm of both sides of Equation (2) (Equation (3)):

$$\ln\sigma_{\mathrm{Tred}} = \ln\left(\frac{1+2.5\varphi}{1-\varphi}\frac{1}{\lambda^{\mathrm{n}}}\right) = \ln\sigma_{\mathrm{Tm}} + B\varphi \tag{3}$$

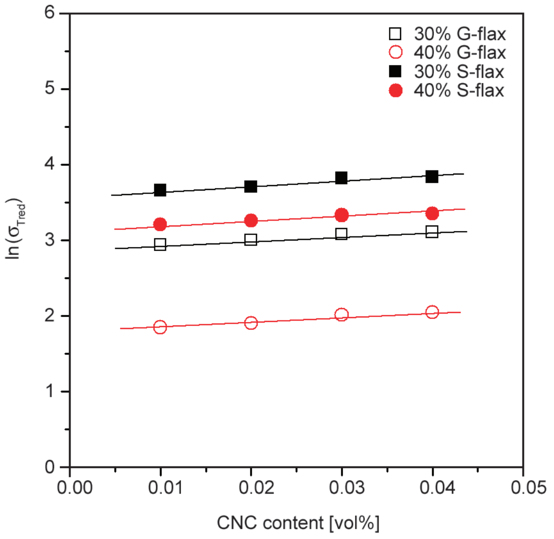

Figure 4. Reduced strength of TPS-CNC films plasticized with glycerol or sorbitol as a function of CNC content. Reduced strength is defined in Equation (3).

The slope of this linear provides parameter B, which is proportional to the extent of reinforcement, and it is determined by the specific surface area of the filler, the strength of interfacial interaction, and the matrix strength [24]. In Figure 4 reduced tensile strength of the composites is plotted against CNC content in the form indicated by Equation (3). Relatively good straight lines are obtained for the four selected cases; standard deviation accounts for the scatter. The slope of the lines gives B values listed in Table 2 together with the goodness of the linear fit, *i.e.*, determination coefficients. The strength of the matrix has a strong effect on the value of B as well [25]. To avoid this effect, parameter B was multiplied by the natural logarithm of the true tensile strength of the TPS [18], and these values ($B\cdot\ln\sigma_{\mathrm{T0}}$) are also placed in Table 1. Analyzing the data in Table 1, it is evident that the nanocellulose produced from flax shows a slightly higher reinforcing effect than the cotton-based CNC, regardless of the amount and type of plasticizer. However, the results of the tests were not sufficiently extensive to warrant any conclusions since the model was tested over a much narrower range for the flax-CNC and the accuracy of the linear fits was also lower than for the cotton-CNC samples. The values of $B\cdot\ln\sigma_{\mathrm{T0}}$, which are proportional to the reinforcing

Table 1. Effect of the matrix polymer (TPS) and the type of CNC on reinforcement.

Fiber	**Plasticizer**	**Plasticizer content [%]**	**Parameter *B***	**$B\cdot\ln\sigma_{T0}$**	**Goodness of fit, R^2**	**Reference**
flax-CNC	sorbitol	30	6.55	23.50	0.9372	Present work
		40	4.93	15.59	0.9634	
	glycerol	30	5.82	16.76	0.9713	
		40	6.98	12.40	0.9637	
cotton-CNC	sorbitol	30	4.87	12.65	0.9968	[18]
		40	4.12	9.64	0.9993	
	glycerol	30	4.92	10.49	0.9952	
		40	4.22	6.90	0.9993	

effect, are similarly dependent on the type and amount of plasticizer for both CNCs. The reinforcing effect of nanocellulose is more significant for TPS containing 30% plasticizer, and the use of sorbitol is more favorable than glycerol.

CNC has the highest reinforcing effect in nanocomposites containing 30% sorbitol. The strength of the interaction between the starch and CNC and the number of interacting molecules also depends on these properties developed in the CNC/plasticizer and starch/plasticizer relationship. The results suggest that the strength of glycerol-starch interactions and the probability of their formation exceeds those of sorbitol. The number of starch-CNC contacts decreases with increasing amounts of plasticizer, as more starch chains can interact with the plasticizer. The reinforcing effect of CNC can be significantly influenced by the aggregation of nanocellulose and the structure of nanocomposite and also by the interaction of water molecules with other components of the system.

3.4. Water vapor sorption and water vapor permeability of nanocomposites

One of the most significant drawbacks of TPS is its highly hydrophilic nature, which can absorb and permeate substantial amounts of water. The question arises whether cellulose nanocrystals, which have very poor accessibility to water molecules and significantly increase the strength of TPS films, can reduce the hydrophilicity and permeability of the polymer. Before investigating the permeability, the water vapor sorption of the nanocellulose films was examined and compared to the TPS films. Figure 5 shows the water vapor sorption of TPS and CNC films containing 30% glycerol and 30% sorbitol as a function of water absorption time. Although the water absorption of CNC films is also significant, it is lower by 20–25% than that of the corresponding TPS film. Consistent with the literature and previous data, sorbitol binds less water than glycerol, so the water absorption of samples made with sorbitol is lower by 5–6% than that of samples prepared with glycerol.

Based on the water vapor sorption data, it could be assumed that CNC can slightly reduce the water vapor permeability of TPS. This effect can indeed be observed in Figures 6. For both the glycerol and sorbitol samples, there is a slight decrease in WVP values regardless of the difference in relative humidity between the two sides of the film (30–100, 50–100, 70–100%). The decrease is slightly larger for the glycerol nanocomposites (Figure 6a). This could be explained by the fact that, for the same concentration of plasticizer, glycerol with a lower molecular weight has a higher mass fraction of end-chain hydroxyl groups, which softens the starch more effectively [26], making the macromolecules more mobile and accelerating the diffusion of water molecules through the film layer under test.

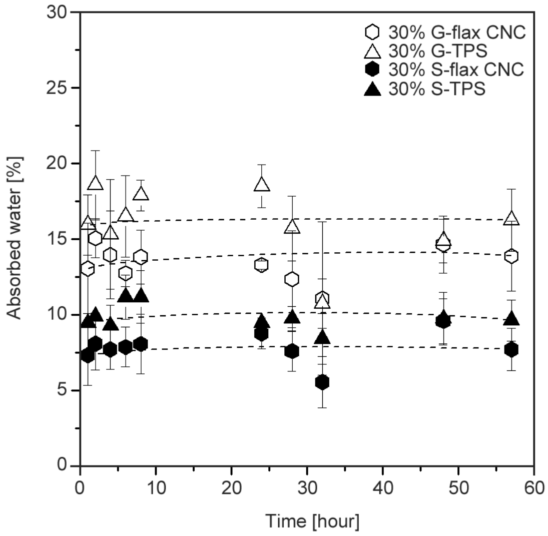

Figure 5. Water vapor sorption of TPS and flax-CNC films plasticized with 30% glycerol (G) or 30% sorbitol (S) and measured at 25 °C and 80% *RH* (Lines are drawn only to guide the eye, and they are not fitted correlations).

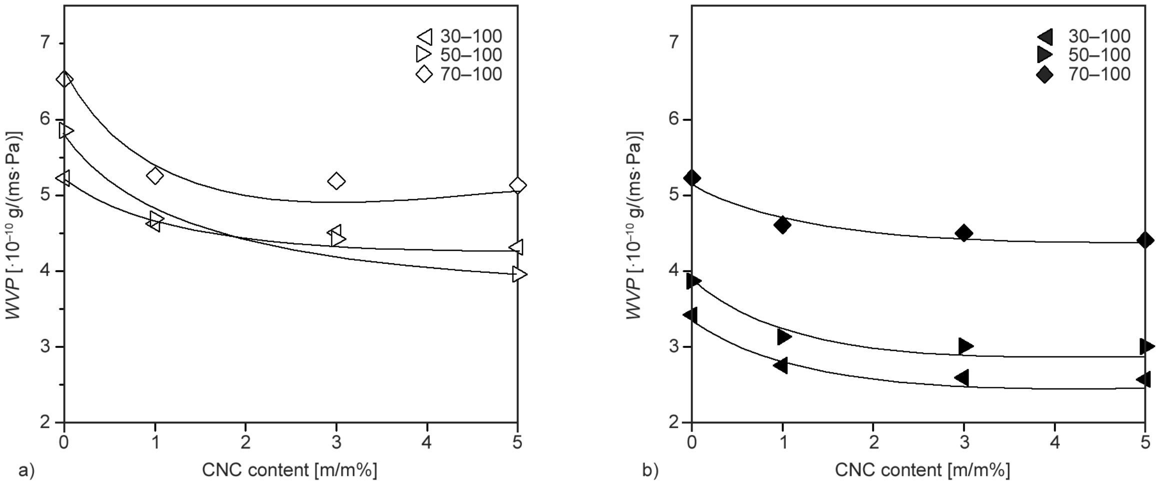

Figure 6. Water vapor permeability of TPS-flax-CNC films plasticized with 30% glycerol (a) or 30% sorbitol (b) as a function of CNC content at different *RH* differences (Lines are drawn only to guide the eye, and they are not fitted correlations).

The water vapor transmission rate (*WVTR*) increased with increasing concentration gradient (from 70–100 to 30–100%), but the permeability varied inversely. The highest permeabilities are obtained at the lowest (70–100%) humidity difference. The most increased water vapor permeability was measured at 70% *RH* of the three external humidity. The higher humidity on the outer side softened the film better than the lower humidity on the outer side since water is the best plasticizer for starch [22]. Water molecules diffused quickly through the films, softened by high moisture on both sides.

3.5. Thermal stability of nanocomposites

Thermal testing was performed on the CNC and TPS films and the nanocomposites containing 3, 10, 20, and 50% CNC. The measurements were carried out to determine whether and to what extent nanocrystalline cellulose reduces the decomposition temperature of the starch film. Indeed, heat more easily degrades the cellulose nanocrystals due to the sulphate ester groups created on their surface during the sulphuric acid treatment used for extraction [27]. Figure 7 shows the weight loss of films as a function of temperature for both plasticizers, while Table 2 summarises the T_{onset} decomposition temperature values, where the large mass loss begins.

From Figure 7, it can be observed that in the case of glycerol plasticizer, the decomposition of the films occurred in several steps. In the initial stage of the measurement, the water decomposes, followed by the glycerol, and the subsequent large slope change

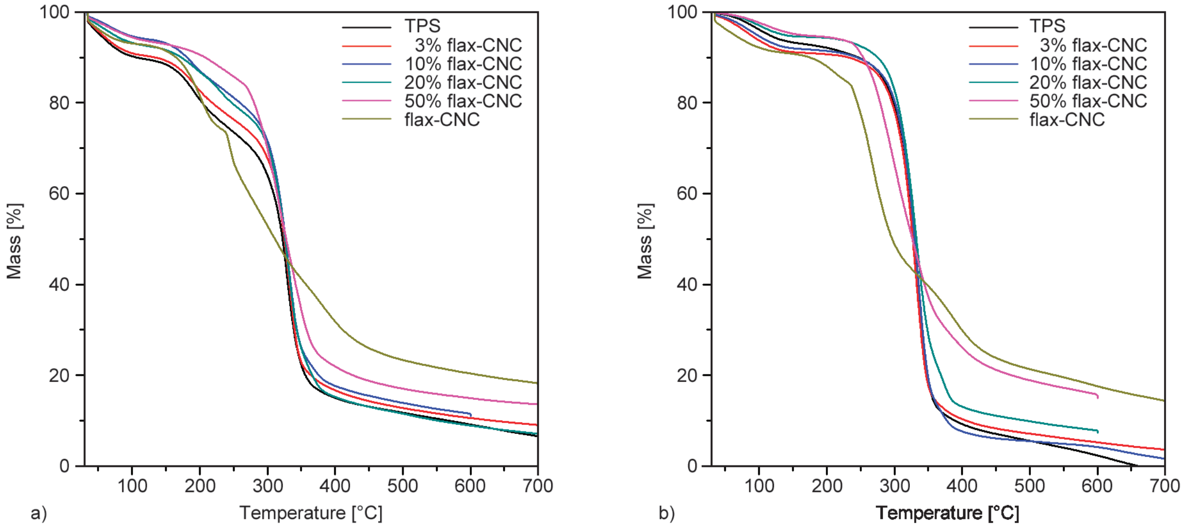

Figure 7. TG curves of TPS, TPS-flax-CNC, and flax-CNC films plasticized with 30% glycerol (a) or 30% sorbitol (b) at different CNC content.

Table 2. The thermal decomposition temperature of the TPS-CNC films plasticized with 30% glycerol or 30% sorbitol at different CNC content.

Plasticizer	CNC content [%]	T_{onset} [°C]
Glycerol	0	307
	3	311
	10	307
	20	309
	50	282
	100	203
Sorbitol	0	305
	3	305
	10	306
	20	301
	50	257
	100	231

belongs to the starch-nanocellulose part. In the case of sorbitol, only the removal of water appears in the first stage. The decomposition of sorbitol does not occur separately.

TGA studies show that up to 20% CNC content, the nanocrystalline cellulose with glycerol plasticizer slightly increases the decomposition temperature of TPS. With sorbitol, however, the CNC has practically no influence on the thermal decomposition of TPS (Table 2). The films containing sorbitol have a few degrees lower decomposition temperatures at 0–20% CNC content than the glycerol-containing samples, but the difference is only a few degrees. Furthermore, the decomposition temperature of the pure CNC films (with glycerol: 203 °C, with sorbitol: 231 °C) and the TPS films containing 50% CNC (with glycerol: 282 °C, with sorbitol: 257 °C) is much lower than that of neat thermoplastic starch films (with glycerol and sorbitol: 307 and 305 °C, respectively). The thermal stability of the sorbitol-containing films is lower than that of the samples prepared with glycerol. The sorbitol-containing samples begin to decompose at a temperature 30 degrees lower. Thus, the difference, in this case, is significant. The 3% CNC concentration, which was the most promising in mechanical tests, does not significantly modify the decomposition temperature. A slightly greater thermal stability was measured for films prepared from pearl millet starch (PMS) with glycerol (30%) and Kudzu CNCs (at 1–7%) than for the neat PMS film [28].

3.6. Retrogradation of TPS in TPS-CNC films

During storage, the plasticized starch can undergo a recrystallization process called retrogradation [29]. Retrogradation modifies several properties (*e.g.*, opacity, hardness, deformability, dimensional stability, *etc.*), affecting the shelf-life and quality of the final products. The rate of retrogradation depends on the glass-transition temperature of TPS, which is determined by the type and amount of plasticizers (glycerol, water) in the polymer [5, 29, 30]. By restraining the mobility of starch molecules with additives, like non-starch polysaccharides or CNC, the retrogradation process can be prevented effectively [6, 31, 18]. X-ray diffraction measurements were carried out three times on the same samples to investigate the effect of prolonged storage (*i.e.*, 1 week, 2 months,

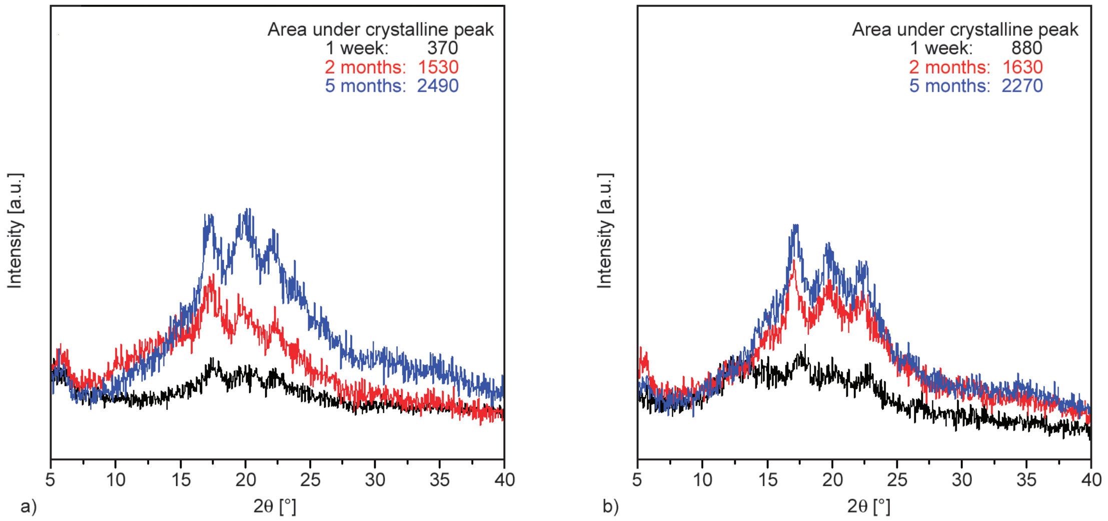

Figure 8. X-ray diffractograms of TPS films containing a) 30% glycerol and b) 3% CNC and 30% glycerol were detected at different times, *i.e.*, after 1 week, 2 months, and 5 months. Changes in crystallinity of TPS and TPS-3% CNC films containing 30% glycerol plasticizer, illustrated by the area under the crystalline peaks.

and 5 months) on the crystal structure evolution. Retrogradation during storage is mainly characteristic of films containing glycerol plasticiser because the efficient plasticizing effect of glycerol makes the starch chains highly mobile and, therefore, easily recrystallized. Figures 8 show the X-ray diffraction curves of the TPS films containing 30% glycerol and the TPS nanocomposite films having 3% CNC and 30% glycerol, respectively, after 1 week, 2 months, and 5 months of storage. The numbers in Figures 8 characterize the areas under the curves defined in the crystalline range of the starch (7–30°). Although recrystallization also occurs in CNC-containing TPS, its extent is slightly smaller than in the pure TPS sample.

4. Conclusions

TPS-CNC composite films were prepared in a wide composition range with glycerol and sorbitol plasticizers used in 30 and 40% concentrations. The CNC was extracted from bleached flax by sulphuric acid hydrolysis. The composites were investigated in detail, and correlations were sought between measured composite properties, CNC properties, and composite composition. The results were compared with those obtained previously for TPS-cotton-CNC composites.

It was clear that the flax-CNC can be used only in low concentrations (0–5%) to produce homogeneous films due to the high aggregation tendency of flax-CNC. The haze index of the composite films increased significantly with increasing CNC content, regardless of the type and amount of plasticizer. Although the CNC increased the strength of the TPS films, its effect was significant only at low CNC content (3% CNC). At higher CNC concentrations, we can achieve no further increase in strength due to the aggregation of nanoparticles. Application of 0–5% CNC decreased water vapor permeability and retrogradation tendency of TPS, in addition to improved mechanical properties. At 0–5% CNC, the thermal stability of the composite films was the same as that of the TPS films, but above 20% CNC caused a significant decrease in thermal stability.

Significant differences were observed in the properties of TPS composites containing CNC from flax or cotton as a function of composition. The differences were mainly due to the aggregation ability of the two types of CNC. Due to the high aggregation of flax-CNC, a given amount of CNC induced a much more significant change in the haziness for TPS-flax-CNC films than for TPS-cotton-CNC films. Furthermore, while the strengthening effect of cotton-CNC was valid over a wide composition range, the flax-CNC increased the strength of TPS only in a narrow concentration range. Nevertheless, the reinforcing effect of the two types of CNCs was influenced by similar interactions. In both cases, the lower plasticizer content and the use of sorbitol as a plasticizer provided favorable conditions to achieve a remarkable reinforcing effect in TPS. The results highlight that precise knowledge of the properties and structure of CNCs used is essential for interpreting the properties of polymer/CNC composites and the compositional dependence of the properties.

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