Effect of cellulose nanocrystals on the properties of pea starch- poly(vinyl alcohol) blend films.

A. Cano\textsuperscript{a}, E. Fortunati\textsuperscript{b,*}, M. Cháfer\textsuperscript{a}, C. González-Martínez\textsuperscript{a}, A. Chiralt\textsuperscript{a}, J.M. Kenny\textsuperscript{b,c}

\textsuperscript{a}Instituto de Ingeniería de Alimentos para el Desarrollo, Universitat Politècnica de València. Camino de Vera s/n 48022 Valencia, Spain.

\textsuperscript{b}Materials Engineering Centre, UdR INSTM, University of Perugia, Strada di Pentima 4, 05100 Terni, Italy.

\textsuperscript{c}Institute of Polymer Science and Technology, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain.

*Corresponding Author. Tel.: +39-0744-492921 Fax: +39-0744-492950, Strada di Pentima, 4, 05100, Terni, e-mail address: HYPERLINK "mailto:elena.fortunati@unipg.it" elena.fortunati@unipg.it (Elena Fortunati)
Abstract

Incorporation of cellulose nanocrystals (CNCs) to pea starch-poly(vinyl alcohol) (PVA) (1:2 ratio) blend films was carried out in order to improve their mechanical and barrier properties and film stability throughout storage, thus overcoming some drawbacks of starch-based films. Different ratios (1, 3 and 5 %) of CNC were used and structural, thermal and physical (barrier, mechanical and optical) properties were analysed in comparison to the control film without CNC. Incorporation of CNC enhanced phase separation of polymers in two layers. The upper PVA rich phase contained lumps of starch which emerged from the film surface, thus reducing the film gloss. CNC were dispersed in both polymeric phases as aggregates, whose size increased with the CNC ratio rise. CNC addition did not implied changes in water vapour barrier of the films, but they became slightly stiffer and more stretchable, while crystallization of PVA was partially inhibited.

Keywords: microstructure, mechanical properties, nanocomposites, optical properties, phase transitions, water vapour permeability.
Introduction

The growing interest in environmentally-friendly materials has promoted research into the development of biodegradable polymers as an alternative to non-biodegradable synthetic petroleum-derived polymers [1, 2]. In response to the consumer requirements for safer and environmentally friendly packaging materials, the combination of biodegradable polymers with bio-based additives has also been analysed to improve the properties of these materials [3]. In this sense, bio-resources obtained from agricultural-related industries have received special attention. Crops fibre components provide a wide range of opportunities for developing new applications in different industrial sectors such as packaging, building, automotive and aerospace industries, electronics, etc. [4].

In the development of packaging materials for food applications, polysaccharides, such as starch, cellulose and their derivatives have commonly been used as film-forming compounds [5]. Cellulose is the most abundant renewable natural polymer resource available in the biosphere [6-8]. It is well known that when cellulose fibres are subjected to acid hydrolysis, the fibres yield defect-free, rod-like crystalline residues [6]. The use of cellulose nanocrystals (CNCs) as fillers in packaging materials has been studied not only because of their interesting physical and chemical properties but also due to their inherent renewability, sustainability and abundance. The most common sources of these nanocrystals include cellulose fibres from cotton, hemp, flax, microcrystalline cellulose, bacterial cellulose [6, 7, 9, 10]. The production of cellulose nanocrystals consists of subjecting pure cellulose material to strong acid hydrolysis under controlled conditions such as temperature, agitation and time, which determine the structure and characteristics of the crystals. Cellulose nanocrystals are used as a reinforcement material due to their large specific surface area (150 m²g⁻¹) [11], surface energy [12] and very high elastic modulus (about 150 GPa) [13]. Moreover, their low density, about 1.566 g cm⁻³ [10], biocompatibility, biodegradability, low energy consumption in manufacturing, and low cost represent remarkable advantages of
cellulose nanocrystals [10, 12, 14, 15] in comparison to others nanomaterials such as nanoclays, SiO₂ and Au nanoparticles for elaboration of low cost nanocomposites. All of this makes cellulose nanostructures to be advantageous bio-based edible additives, which are able to enhance the bio-polymer performance, in terms of the mechanical, thermal and barrier properties [2, 8]. However, cellulose nanocrystals have some drawbacks, such as the difficulty to disperse homogenously in the polymer matrix [14], as a result of their agglomeration into flakes during film formation. Due to the hydrophilic character of cellulose nanocrystals, the main technique employed to transfer them from an aqueous dispersion into an organic polymer has been the casting-evaporation process [6].

Biopolymer nanocomposites are the result of the combination of biopolymers and nanoparticles of inorganic/organic fillers [15]. Cellulose nanocrystals have been incorporated into a wide variety of biopolymer matrices including (poly)caprolactone [16], carboxymethyl cellulose [17], (poly)vinyl alcohol [7, 8, 10, 15], (poly)lactic acid [9, 14], chitosan [18], starch [5, 19] and biopolymer blends, like poly(lactic acid)-poly(hydroxybutyrate) [20, 21]. In general, the hydrophilic nature of both biopolymer and nanocrystals leads to excellent interfacial compatibility, resulting in enhanced mechanical and thermal properties of the composite material [5, 15].

The use of starch as a biopolymer matrix in combination with other polymers to reduce the starch film drawbacks (poor mechanical and water vapour barrier properties) has been explored by different authors [22, 23]. The incorporation of PVA into gelatinized pea starch matrices implied the formation of interpenetrated polymer networks. In fact, the blend films showed beneficial effects on the mechanical properties of the films, these becoming much more extensible and stable during storage, and on water barrier properties [24]. Different studies into starch-PVA blends have been carried out, focusing on their biodegradability [25, 26] and the effect of the incorporation of different additives to the blends. The effect of adding citric acid [26], glutaraldehyde [27], urea [28], calcium chloride [29], or nanoparticles like nano-sized poly(methyl methacrylate-
co-acrylamide) particles [30] on PVA-starch blend properties has been analysed for different aims (compatibility enhancement or development of biomedical and packaging materials). Nevertheless, no studies have been found into the effect of cellulose nanocrystals on the properties of pea starch-PVA blend films. The aim of the present work was to study the effect of incorporating cellulose nanocrystals into pea starch-PVA blend films in terms of their nano- and microstructure, thermal behaviour and physical properties at different ageing times.

### 2. Materials and Methods

#### 2.1 Materials

Pea starch (S) was purchased from Roquette Laisa España S.A. (Benifaó, Valencia, Spain), poly(vinyl alcohol) (PVA) (M<sub>w</sub>: 89,000-98,000, hydrolysed, +99%, viscosity: 11.6-15.4 cP, 4% in H<sub>2</sub>O at 20 °C), microcrystalline cellulose (MCC) (powder, 15 - 20 µm) were from Sigma Aldrich Química S.L. (Madrid, Spain). Glycerol and magnesium nitrate-6-hydrate (Mn(NO<sub>3</sub>)<sub>2</sub>) were supplied by Panreac Química S.A. (Castellar de Vallès, Barcelona, Spain). Sulphuric acid, ion resin (Dowex Marathon MR-3 hydrogen and hydroxide), Whatman 541 filter paper and NaOH were also purchased from Sigma Aldrich Química S.L. (Milan, Italy).

#### 2.2 Extraction of Cellulose nanocrystals (CNC)

A suspension of cellulose nanocrystals (CNC) was prepared from microcrystalline cellulose (MCC) by hydrolysis using sulphuric acid, 64% (wt/wt) at 45 °C for 30 min, as previously reported by Cranston and Gray [31] and Fortunati [9]. Immediately following the acid hydrolysis, the suspension was diluted 20 fold with deionized water and maintained at rest overnight. Afterwards, the dispersion was centrifuged at 4,500 rpm for 20 min to separate the cellulose crystals. The precipitate was dialyzed against deionized water for 5 days and then neutralized with mixed bed ion resin for 48 h.
Afterwards, the suspension was filtered through filter paper. The CNC filtrate was neutralized by adding 1.0 % (v/v) of 0.25 M NaOH. Finally, the CNC dispersion was homogenized by ultrasonic treatment, using a tip sonicator (Vibracell, 750 Sonics & Materials, Inc., Newton, USA) for 10 min in an ice bath. The dry matter content of the CNC dispersion was determined by the drying oven method [32], giving 11.1±0.2 %. This value was a little low compared with that reported by other authors [21]. CNC obtained nano-crystals showed dimensions ranging from 100 to 200 nm in length and 5 to 10 nm in width, according to FSEM observations [10].

2.3. Preparation of films

Films were obtained by casting from film forming dispersions (FFDs). Starch (1 %w/w) was dispersed in an aqueous solution at 90 ºC for 30 min with continuous stirring to induce starch gelatinization. Thereafter, the dispersion was homogenized using a rotor-stator homogenizer (Ultraturrax D125, Janke and Kunkel, Germany) at 13,500 rpm for 1 min and 20,500 rpm for 3 min. Immediately following the starch gelatinization, PVA was dispersed in the aqueous solution in a S-PVA ratio of 1:2 (w/w), and maintained at 90 ºC for 30 min until complete dissolution. When the dispersion was cooled, 0.25 g of glycerol per g of starch was added on the basis of previous studies [33]. This FFD was used to obtain the control films. Cellulose nanocrystal dispersion was homogenized with the polymer FFDs by means of a tip sonicator for 4 min in ice bath. Different CNC mass ratios were considered: 0, 1, 3 and 5 g of CNC per 100 g of total polymers (sample codes: C, 1 %, 3 % and 5 %, respectively), considering the dry weight of CNC and polymers.

To obtain the films, the FFDs were poured into Petri dishes, in a proper amount to provide a surface density of solids of 145 g m⁻². Films were dried at 40 ºC in a convection oven for 48 h and afterwards, peeled off the casting surface and conditioned at 53 % RH, using magnesium nitrate-6-hydrate (Mn(NO₃)₂) saturated solution at room temperature (≈ 25 ºC) until further analysis. The film thickness was
measured at six random positions with a calliper (MicrometerStarrett) to the nearest 0.001 mm.

2.4. Characterization of films

2.4.1. Microstructure

Microstructural analysis of films was carried out using both a field emission scanning electron microscope (FESEM) (Supra™ 25-Zeiss, Germany) and an atomic force microscope (AFM) (Multimode 8, Bruker AXS, Inc. Santa Barbara, California, USA), with a NanoScope® V controller electronics. To this end, two replicates per formulation were observed. FESEM observations were carried out on the film surface and on their cross section. To prepare the cross section samples, films were frozen in liquid nitrogen and cryofractured. Afterwards, samples were gold coated, and observed using an accelerating voltage of 2 and 5 kV, for the surface and cross-section observations, respectively.

The surface morphology was also analysed using AFM. The resulting data were transformed into a 2D image. Measurements were taken from 50 x 50 μm and 3 x 3 μm areas of the film surface, using the phase imaging mode.

AFM with the peak force QNM (Quantitative NanoMechanics) mode was also used to analyse the film surface nanostructure. Measurements were taken from 20 x 20 μm areas of the film surface and the resulting data were transformed into a 2D image (DMT modulus map).

2.4.2. Fourier Transform Infrared (FTIR) spectroscopy

FTIR spectra of the films were obtained by a Jasco FT-IR 615 spectrometer, (Easton MD, USA) in transmission mode, in the range of 400-4000 cm⁻¹. A few drops of different film forming dispersions were cast on silicon plates, after which they were dried and measured. Each sample was characterized in duplicate.
2.4.3. Thermogravimetric analysis

Thermal weight loss (TG) and its derivate (DTG) of film samples vs. temperature were obtained using a thermogravimetric analyzer (Seiko Exstar 6300, Italy). In the test, samples were heated from 30 °C to 600 °C at 10 °C min⁻¹, using a nitrogen flow. Prior to the analyses, samples were conditioned for 1 week. Thermal degradation temperatures (the maximum of the DTG curves (T_{mp}) and secondary degradation temperature peak (T_p)) were obtained. Measurements were taken in triplicate.

2.4.4. Differential scanning calorimetry

Differential scanning calorimeter (DSC) (TA Instrument, Q200, USA) was used to analyse phase transitions in the films as a function of the temperature. Measurements were carried out in triplicate under nitrogen flow in the temperature range −25 to 230 °C, at 10 °C min⁻¹, by performing three scans: First, samples were heated from room temperature to 230 °C and maintained for 5 min at 230 °C. Then, samples were cooled down to −25 °C and heated again until 230 °C. Data were recorded both during the cooling and second heating steps. From thermograms of the cooling step, the crystallization temperatures (T_c) and enthalpy (ΔH_c) values were obtained. From the second heating step, glass transition temperature (T_g), melting temperature (T_m) and melting enthalpy (ΔH_m) values were obtained. Prior to the analyses, samples were conditioned for 1 week.

The crystallinity degree of PVA was calculated as shown in equation 1:

\[ X = \frac{1}{X_{\text{PVA}}} \left[ \frac{\Delta H}{\Delta H_0} \right] \times 100 \]  

(1)

Where \( \Delta H \), is the melting enthalpy of the sample (expressed in J g⁻¹ PVA), \( \Delta H_0 \), the melting enthalpy of a 100% crystalline PVA sample (161.6 J.g⁻¹ [34]) and \( X_{\text{PVA}} \), the mass fraction of PVA in the film.
2.4.5. Moisture content

Film moisture content (MC) was analysed by drying the film samples in a vacuum oven at 60 ºC for 24 h. Later on, the pre-dried samples were placed in desiccators containing P₂O₅ until reaching a constant weight. Five replicates per film formulation for one and five weeks were analysed.

2.4.6. Water vapour permeability (WVP)

Water vapour permeability (WVP) was evaluated in films equilibrated for 1 and 5 weeks, following the gravimetric method ASTM E96-95 [35] by using Payne permeability cups (Payne, elcometer SPRL, Hermelle/sd Argenteau, Belgium) of 3.5 cm diameter. Deionised water was used inside the testing cup to achieve 100 % RH on one side of the film, while an oversaturated magnesium nitrate solution was used to control the RH on the other side of the film. A fan placed on the top of the cup was used to reduce resistance to water vapour transport. Water vapour transmission rate measurements (WVTR) were performed at 25 ºC. To calculate WVTR, the slopes in the steady state period of the weight loss vs. time curves were determined by linear regression. WVP was calculated according to [36]. For each type of film, WVP measurements were taken in quadruplicate.

2.4.7. Mechanical properties

Mechanical properties were measured using a Universal Test Machine (Digital Lloyd instrument, West Sussex, UK), following the UNI ISO 527-1 [37], by using 5 mm min⁻¹ and a load cell of 1.5 N. Equilibrated film samples (1 x 5 cm) for 1 and 5 weeks were mounted in the film-extension grips (A/TG model), which were set 20 mm apart. Stress-Hencky strain curves were obtained and the tensile strength at break (TS), percentage of elongation at break (Ɛ) and elastic modulus (EM) were calculated. Measurements were taken at room temperature with eight replicates per formulation.
2.4.8. Ultraviolet-visible spectrophotometry

Film samples equilibrated (1 x 1 cm) for 1 and 5 weeks were analysed by means of a UV–VIS spectrophotometer (Perkin Elmer Instruments, Lambda 35, Waltham, USA), by using a wavelength range between 250 and 1000 nm.

2.4.9. Internal transmittance

Internal transmittance (Ti) as a measure of the transparency of the films was determined through the surface reflectance spectra in a spectrocolorimeter CM-3600d (Minolta Co, Tokyo, Japan) with a 30 mm illuminated sample area by applying the Kubelka–Munk theory for multiple scattering to the reflection spectra, following the methodology described by Cano, et al. [36]. Measurements were taken in triplicate in films equilibrated for 1 and 5 weeks.

2.4.10. Gloss

Gloss was measured using a flat surface gloss meter (Multi- Gloss 268, Minolta, Langenhagen, Germany) at an incidence angle of 60º, according to the ASTM standard D523 [38]. Prior to gloss measurements, films were conditioned for 1 and 5 weeks. Gloss measurements were performed in triplicate. Results were expressed as gloss units, relative to a highly polished surface of standard black glass with a value close to 100.

2.4.11. Overall migration

Overall migration tests in films conditioned for 1 week were carried out by following current legislation [39, 40]. Rectangular film strips of 20 cm² total area were immersed in a glass tube with 20 mL of food simulants (ethanol 10 % (v/v) - simulant A- (Sigma Aldrich Química S.L., Milan, Italy) and isooctane - simulant to D2- (Sigma Aldrich Química S.L., Milan, Italy)), keeping the established relation of 6 dm² kg⁻¹. Samples in simulant A were kept in a controlled chamber at 40 ºC for 10 days, while samples in
iso-octane were kept at 20 °C for 2 days. After the incubation period, the films were
removed and simulants were evaporated to dryness. Afterwards, the residue was
weighed with ±0.001 mg precision in order to determine the overall migration value in
mg kg⁻¹ of simulant. For each sample, three determinations were carried out.

2.5. Statistical analysis

Results were analysed by analysis of variance (ANOVA), using the Statgraphics Plus
5.1. Program (Manugistics Corp., Rockville, MD). To differentiate samples, Fisher’s
least significant difference (LSD) was used at the 95 % confidence level.

3. Results and discussion

3.1. Nano- and micro-structure of the films

Figures 1 and 2 show the FESEM micrographs of the surfaces and cross sections of
the different films, respectively. Control films showed phase separation of starch and
PVA due to the lack of polymer compatibility, according to what was previously
observed by other authors [24, 41]. Surface of control films shows the formation of
globular structures which can be attributed to domains of one of the polymeric phases
dispersed in the continuous phase of the other. When CNC nanocrystals were
incorporated into the film formulation, the surface concentration of dispersed domains
increased, this being more marked for the highest CNC content (5 %).

The cross section micrographs of control films (Figure 2) showed two interpenetrated
networks of both polymers where the crystalline zones of PVA can also be appreciated.
In films containing CNCs the formed two layers in the films are more clearly
differentiated. The top phase corresponds to about one third of the film thickness,
according to the ratio of starch to PVA, which suggest that starch rich phase mostly
separated at the top of the films whereas PVA predominate in the down layer. The top
layer generally shows a less smooth appearance, showing the coexistence of PVA
dispersed domains in a more continuous starch matrix. Some of them emerged to the
film surface, as shown in the surface micrographs (Figure 1). The PVA rich phase also shows lumps of starch phase. Distribution of nanoparticles in the different phase cannot be clearly appreciated at the magnification level of micrographs, although the PVA phase shows a more granular aspect which could indicate that nanoparticles could be present in this phase to a greater extent. Micrographs at higher magnification allow us to appreciate this effect. For the highest ratio of CNCs (5 %), these appear distributed in both phases, thus modifying their general appearance. The aggregation of CNC in some film areas could be observed, which is due to their strong hydrogen bonding capacity. Khoshkava and Kamal [12] also reported that at higher CNC concentration CNC aggregation occurs to a great extent.

Figure 3 shows AFM images of control film and those containing CNC, obtained by using Phase Imaging mode derived from Tapping Mode. Raw data were converted into 2D images and their scale is expressed as degrees. Phase Imaging allows to detect variations in composition, adhesion, friction, viscoelasticity and other properties in the material surface at nano-scale level, providing material property contrast. Surface of 50 µm² of control films shows two different phases in agreement with that observed in the surface FESEM images. The dispersed phase in the control film corresponds to emerging PVA lumps in the starch continuous phase of the upper layer of the films. Nevertheless, the dispersed phase concentration at the film surface increased when CNC ratio rose in the formulation. Observations a higher magnification (areas of 3 µm²) were carried out on the continuous and dispersed surface phases to observe possible location of CNC at the film surface. These images are shown in Figure 3. For 1 % of CNC, no evidences of the nanocrystals in any phase are detected, probably due to their low ratio in film formulation. Nevertheless, at 3 % and 5 %, CNCs were observed in both, dispersed and continuous polymer phases. At 3 % of CNC, great aggregates of particles are present in the continuous phase (mean size 200 nm) whereas particles are better dispersed in the PVA dispersed phase. At 5 % CNCs, particle aggregation is more accused appearing as enlarged formations whose perimeter is completely
covered by flocculated nanocrystals. This formations appeared in both PVA and starch phases at the surface. As reported by Arrieta et al. [21] the greater the CNC concentration, the higher the aggregation level in the system. Differences in the surface mechanical resistance were observed by means of AFM in Peak Force QNM mode (Figure 4). The maps of Log DMT modulus for control films revealed the two phases previously mentioned at surface level, but no great differences in mechanical resistance between both could be detected. In 1 % CNC film formulation, similar values of log DMT modulus to those of control films were observed, probably due to low concentration of CNC in the observed area, as deduced in phase imaging. The incorporation of 3 % and 5 % of CNC gave rise to much higher differences in the values of DMT modulus of a given surface, especially for 3 %. In this case, the hardest areas are particulate in shape, which agrees with the greater hardness of crystalline structure of dispersed CNC. At 5 %, a high proportion of very soft small areas can be observed, which can be attributed to voids left by the aggregates of CNC which probably are separated from the surface by the cantilever during the test, in part due to their big size, despite the images reveals good interfacial adhesion of CNC to the pea starch-PVA matrix. Figure 5 shows FTIR spectra for control and nanocomposite films, showing the wavenumber values corresponding to the main peaks in each sample. The spectrum of the control film showed several characteristic peaks of stretching and bending vibrations of groups of starch and PVA chains. The broad band located between 3200-3600 cm\(^{-1}\) corresponds to the stretching vibration mode of hydroxyl groups from the absorbed water and from the polymers themselves, [10, 42, 43]. The relative intensity of this band decreased when the ratio of CNC increased in the films. The peak at around 2940 cm\(^{-1}\) is related with alkyl groups, C-H stretching [10, 43, 44] and it increased in intensity as the CNC ratio increased, which can be explained by the contribution of C-H vibration in the crystalline structures. The peak at 1645 cm\(^{-1}\) corresponds to the H-O-H group deformation [10] and it appears better resolved in
films with CNC. The peaks associated with the bending vibration mode of hydroxyl
group appear at around 1420 cm\(^{-1}\) and they show a slight displacement of 20 units with
respect to the control film when CNC are present in the matrix, while an increase in
intensity and resolution of this peak was observed when CNC ratio increased up to 3
%. The stretching vibration of C-O in the C-C-O group and in the starch glucose ring
corresponds to the peaks at 1032 and 854 cm\(^{-1}\), respectively [10]. These bands also
suffered changes due to the presence of CNC in the films.

The addition of CNC, especially at 3 and 5 %, resulted in a slight reduction of the
intensity of the -OH stretching band, a widening of band at 2940 cm\(^{-1}\) due to C-H
stretching, the appearance of an additional peak at 1733 cm\(^{-1}\), assigned to the C-C-O
stretching, and changes in the peaks resolved between 850 and 1670 cm\(^{-1}\). In this
sense, it is remarkable that the C-OH bending vibrations of alcohol groups present in
 cellulose appear at 1100 cm\(^{-1}\) [10]. The slight changes introduced by CNC in the FTIR
spectra of PVA-starch films suggest the interactions between hydroxyl groups (-OH) on
the CNC surface and the –OH of the polymer blend chains, as proposed by other
authors [50].

3.2. Thermal properties of the films

DSC and TGA measurements were used to study the thermal behaviour of the films, in
order to know phase transitions and the thermal stability of the materials [45] as
affected by the addition of CNC at different contents.

Table 1 shows the results obtained from the DSC analysis. The PVA crystallization
pattern showed one secondary peak at about 144 °C and a main peak located around
201 °C. Secondary peak appears at lower temperatures (supercooling) due to kinetic
hindrances attributable to the low mobility of the polymer chain segments at the end of
the crystallization process. The melting temperature (Tm) of control films was 227 °C,
without the split observed in crystallization. No significant effect of the CNC incorporation was observed in the Tm values, as previously found by Habbi et al. [6].

Crystallization enthalpy (Table 1), expressed as J g\(^{-1}\) of PVA, showed a certain degree of variability and there were no significant differences among samples. The average value was 70 J g\(^{-1}\) of PVA, slightly lower than the melting enthalpy value, which indicates that supercooling occurred during the cooling step. Values of melting enthalpy, expressed in J g\(^{-1}\) of PVA, reveal that PVA crystallization was partially inhibited by the presence of CNC, since the \(\Delta H_m\) value decreased as the CNC ratio in the films rose. In fact, the degree in crystallinity of PVA (X in Table 1) was reduced by about 50 %, with respect to the control film, when 5 % of CNC was added. However, Rescignano et al. [15] observed that the crystallinity increases slightly with the addition of cellulose nanocrystals in PVA films, although their reported values are much smaller (15 %) as compared with the obtained values in this study (close to 70 % in control film). The CNC inhibition effect in PVA crystallization is also deduced from the greater supercooling observed in the cooling scan for samples containing CNC.

Glass transition observed in the films must be assigned to the PVA phase bearing in mind the temperature range where it occurs, while this transition was not detected for the starch phase, due to its lower ratio in the film. Our previous studies [24] on PVA-starch blend films found the Tg values at 124±2 °C and at 76±4 °C, respectively for the starch and PVA phases. Tg values were taken from the heating step when crystallization of PVA is completed and the amorphous phase contains the non-crystallized fraction. In all cases, the values obtained in the cooling step were slightly lower, which indicates that the mean molecular weight of the amorphous fraction is lower when crystallization was not completed. Therefore, this would point to the fact that the longer chains crystallize prior to the shorter ones. The obtained Tg value of PVA in the control films was 79 °C, which was similar to that reported by other authors for PVA films [10, 15]. The incorporation of CNC to the films provoked a decrease of about 2 °C, which can be related with the partial inhibition of the PVA crystallization.
(especially the shorter chains, as commented on above) and the subsequent decrease of the mean molecular weight of the amorphous PVA fraction. Other authors [6, 9, 15] did not found changes in the glass transition temperature of the polymer (PLA and PVA) when cellulose nanocrystals were incorporated to the matrix.

DSC analysis reveals that PVA crystallization was partially inhibited when CNC are present as filler in the blend films, this effect being more marked when they contain 5 % of nanocrystals. The lack of crystallization gave rise to a decrease in the Tg of the amorphous phase which suggests that the shorter PVA chains remain in the amorphous phase.

Figure 6 shows the weight loss (TG curve) and its derivate (DTG curve) as a function of the temperature for control films and formulations containing cellulose nanocrystals. The temperatures for the main degradation steps of the films are summarized in Table 1. For control films, three weight loss steps were observed. Similar multi-step weight loss behaviour was described for PVA films [7, 15] and for corn starch-PVA blend films obtained by casting [40]. The initial weight loss, up to about 90 ºC, can be attributed to the loss of bonded water in the film [7, 8, 27] with total weight loss in this range of about 10 %. The second step, between 150-380 ºC, is related to the main degradation process (peak temperature 347 ºC), in which dehydration reactions, followed by polymer scission and decomposition, take place. Total weight loss in this range is about 70 %. Moreover, in this step the acetyl groups of PVA were transformed into acetic acid molecules and successive catalytic degradation of the main chain by in situ stripping at higher temperatures occurs [15, 46]. The third step takes place at between 380 – 500 ºC and it can be attributed to the degradation of the by-products generated by PVA during the thermal process [7, 23]. In previous studies, it was observed that in pea starch films only two weight loss steps occur: the loss of bonded water up to 100 ºC and the main degradation at 315 ºC.

The addition of cellulose nanocrystals into the films led to a similar weight loss pattern to the control films, showing the three weight loss steps commented on above (Figure
6). There were no notable changes in the pattern of thermal degradation of nanocomposites or in the temperature of the main peaks (Table 1), except for films with 5 % CNC, where a slight decrease in $T_{mp}$ was observed. Likewise, the temperature of the secondary peak was slightly higher due to the influence of cellulose thermal behaviour [15].

3.3. Physical properties of the films

The analysis of the physical properties of studied films was carried out to know their barrier, optical and mechanical behaviour. Film thickness was 98±8 $\mu$m for all formulations. Table 2 shows the water vapour permeability values (WVP) of the films at 25 °C and at a 53-100 % RH gradient, together with their equilibrium moisture content and optical properties after 1 and 5 storage weeks. After 1 week, the moisture content was slightly lower for samples containing 3 % and 5 % CNC, but their value increased throughout 5 storage weeks, reaching a similar value in all cases in the range 6-7 %. This suggests that CNC limit the moisturising rate till sample equilibration, despite the hydrophilic character of these nanoparticles [6, 10], probably due to the structural changes induced in the films and the increase in the tortuosity factor in the matrix associated with the presence of the dispersed nanoparticles.

The WVP values of the films must be as low as possible to efficiently limit the water vapour transfer when it is in contact with food systems [19]. Mean values of studied films ranged between 3.1-3.6 g.mm.kPa$^{-1}.h^{-1}.m^{-2}$ and no notable differences are found among formulations or due to the storage time. Nevertheless, as deduced from the moisture equilibration time, CNC seem to slightly reduce WVP values (increase in the tortuosity factor for mass transfer), but to a very limited extent, probably due to their high water affinity which contributes to the hydrophilic character of the matrix and to solubility of water molecules, thus enhancing water transport.
Optical properties, UV-VIS spectra, transparency (Ti) and gloss of the films are directly related with their nano- and micro-structure. The UV-VIS spectra of control and cellulose nanocrystals based films in the UV range are shown in Figure 7, where the greatest differences were observed. The films exhibit higher values of transmittance (T) in the visible light range (400-800 nm) than in the UV range (200-400 nm), according to Chen et al., [42] for PVA films. The control films exhibited values of transmittance above 90 % in the visible light range, whereas the values were much lower in the UV range. The addition of cellulose nanocrystals provoked a decrease in film transmittance over the whole UV-VIS range, but this is more marked in the UV range, where T values decreased by about 60 % for the films with the highest ratio of nanocrystals. These results confirm the greater opacity to UV radiation of nanocomposites observed by other authors [10, 20], which represents an advantage in terms of the food protection against oxidative processes or other UV induced reactions. In terms of transparency to visible light, the internal transmittance (Ti) at 450 nm (Table 2) reveals a small progressive increase in opacity as the CNC ratio rose in the films, regardless of the storage time, in agreement with the rise in the concentration of the nanocrystal dispersed phase. As concerns the film’s gloss, they showed very low values at 60º incidence angle, as compared with the gloss values of pure pea starch or PVA films (27 and 150 units, respectively, data not reported). This can be attributed to the surface roughness of the films where lumps of the starch-rich phase are dispersed in the continuous PVA-rich phase, as discussed above. This provoked irregularities at the film surface which contributes to light dispersion, giving a matt appearance. The incorporation of CNC did not significantly affect the gloss of the films.

The mechanical behaviour of the films is shown in Figure 8 where the typical stress-strain curves of control blend (C) and CNC composite films 1 %, 3 % and 5 %, after 5 weeks of storage time are shown. The different tensile behaviour of control films and composites can be clearly observed. The presence of CNC affected the film extensibility; the higher the CNC ratio, the more stretchable the material. Similar
behaviour has been reported by several authors [8, 20] for other films containing CNC.

It is highlighted that the extensible response of films is closely related to the different concentrations of nanocrystals in the matrix, determining the volume fraction of the reinforcement, the dispersion degree in the matrix, and the interactions between the nanocrystals and the polymers [15].

Elastic modulus (EM), tensile strength at break (TS) and percentage of elongation at break (Ɛ %) are used to describe the mechanical behaviour of films. Table 3 shows the mean values of these parameters for control films and composites. The obtained values are coherent with those reported by other authors for pea or corn starch-PVA films [24, 28, 30]. Cellulose nanocrystals improved the mechanical behaviour of films in terms of their stretchability, without decreasing their resistance to break (p<0.05), while the elastic modulus tends to increase slightly. After 5 weeks’ storage time, a similar behaviour was observed for both blend and composite films: the resistance to break is reduced, as well as the elastic modulus, which can be attributed to the moisture gain of the films during storage, which makes the network cohesion forces weaker. Nevertheless, at this time, the reinforcement effect of CNC was more evident since the elastic modulus of composites containing 3 or 5 % CNC was higher.

The mechanical impact of CNCs on blend films can be, in part, explained by the limitation of crystallization of PVA, but also by the formation of a percolating network within the polymer matrix, as reported by other authors [6]. In this network, the stress is assumed to be transferred through crystal-crystal interactions and crystal-polymer matrix interactions [10]. According to Favier et al. [47], the critical percolation volume fraction (percolation threshold: Xc) can estimated from statistical percolation theory for cylindrically shaped particles taking into account their aspect ratio (A) by the relation: (Xc=0.7/A). For composite films with CNCs, A can be estimated as 20 and Xc is 0.035. So, percolation threshold was reached for all composites and percolation network formation can explain the enhancement of mechanical behaviour of the films.
PVA-starch films containing cellulose nanocrystals were more stretchable and stiffer with comparable resistance to break than pea starch-PVA films and so, they can be considered more adequate for food packaging applications.

Overall migration tests with simulants were carried out to determine the total amount of non-volatile substances that might migrate into foodstuffs from film matrices [48] for the purposes of checking whether they meet the migration limit (60 mg kg⁻¹ simulant) established by current legislation [39]. Table 4 shows the obtained values of overall migration for control and nanocomposite films in both food simulants. After 20 days in ethanol 10 % (v/v) simulant, no significant differences in overall migration (p ≤ 0.05) between control and composite films were found, except for films with 5 % of CNC which showed higher values, although well below the established limit. However, after 2 days at 20 ºC in isooctane, the maximum migration level was reached for the control films, although migrated amounts are in the order of 1000 times lower than that obtained in the polar simulants. The different migration behaviour of control and nanocomposites in the two food simulants reveals the greater hydrophilic nature of films containing CNC, as reported by Fortunati, et al. [49]. The CNC addition significantly decreased (p<0.05) the amount of material that migrates to non-polar food simulants, while increasing the migrated amounts in polar simulants. Therefore, CNC make the films more adequate for applications in high fat content foods.

Conclusions

The pea starch-PVA blend films showed phase separation and CNC are distributed in both, starch rich phase and PVA rich phase. They are present as aggregates of different sizes depending on their ratio in the film; the higher the ratio, the greater the aggregates, as deduced from the AFM analysis at surface level. No changes in water vapor permeability occurred due to the presence of CNC, despite the increase in the hydrophilic nature of the films revealed by the overall migration values in polar and non-polar food simulants. Films with CNC became slightly stiffer and more stretchable.
than control films, while crystallization of PVA was partially inhibited by CNC addition. The improvements conferred by CNC in mechanical properties of pea starch-PVA blend films make them more adequate for food applications, especially for high fat foods, where overall migration values were very low.

Acknowledgments
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REFERENCES


[40] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.


Table 1: Thermal properties of control blend films and those containing 1, 3 and 5 %wt of CNC obtained by DSC and TGA analysis. Mean values ± standard deviation.

<table>
<thead>
<tr>
<th>FILMS</th>
<th>Cooling</th>
<th>Heating</th>
<th>TGA analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tc1 (ºC)</td>
<td>Tc2 (ºC)</td>
<td>ΔHc (J.g⁻¹pva)</td>
</tr>
<tr>
<td>C</td>
<td>143.8±0.9a</td>
<td>200.7±0.1a</td>
<td>65±6a</td>
</tr>
<tr>
<td>1 %</td>
<td>135±4a</td>
<td>201.1±1.2a</td>
<td>74±8a</td>
</tr>
<tr>
<td>3 %</td>
<td>141±3b</td>
<td>201.2±1.2a</td>
<td>71±6a</td>
</tr>
<tr>
<td>5 %</td>
<td>145.9±0.1ab</td>
<td>202.3±0.2a</td>
<td>60.7±0.2a</td>
</tr>
</tbody>
</table>

Tc: crystallization temperature; Tm: melting temperature; Tg: glass transition temperature; ΔHc: enthalpies of crystallization; ΔHm: enthalpies of melting. X: percentage of crystallinity; Tmp: main peak temperature and Tp: temperature of second degradation peak.

ab different letters in the same column indicate significant differences among formulations (p<0.05).
Table 2: Moisture content (MC), water vapour permeability (WVP), internal transmittance (Ti) at 450 nm and gloss values at 60º of control films and those containing 1 %, 3 % and 5 % of CNC, after 1 (1W) and 5 (5W) storage weeks. Mean values ± standard deviation.

<table>
<thead>
<tr>
<th>FILMS</th>
<th>MC (%d.b.)</th>
<th>WVP (g.mm.kPa⁻¹h⁻¹m⁻²)</th>
<th>Ti (450nm)</th>
<th>Gloss 60º</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1W</td>
<td>5W</td>
<td>1W</td>
<td>5W</td>
</tr>
<tr>
<td>C</td>
<td>6.6±0.8a¹</td>
<td>6.7±0.3ab¹</td>
<td>3.5±0.3a¹</td>
<td>3.41±0.15a¹</td>
</tr>
<tr>
<td>1 %</td>
<td>6.4±0.5b¹</td>
<td>6.9±0.2a¹</td>
<td>3.4±0.3a¹</td>
<td>3.6±0.3a¹</td>
</tr>
<tr>
<td>3 %</td>
<td>4.73±0.07c¹</td>
<td>6.5±0.3b²</td>
<td>3.43±0.15a¹</td>
<td>3.25±0.14a¹</td>
</tr>
<tr>
<td>5 %</td>
<td>4.5±0.2c¹</td>
<td>5.8±0.2c²</td>
<td>3.2±0.3a¹</td>
<td>3.07±0.66a¹</td>
</tr>
</tbody>
</table>

a,b,c different letter in the same column indicate significant differences among formulations (p<0.05).

1,2 different number in the same file indicate significant differences among storage time (p<0.05).
Table 3: Values of elastic modulus (EM), tensile strength at break (TS) and percentage of elongation at break (Ɛ, %) of control blend films and those containing 1 %, 3 % and 5 % of CNC, after 1 (1W) and 5 (5W) storage weeks. Mean values ± standard deviation.

<table>
<thead>
<tr>
<th>FILMS</th>
<th>EM (MPa)</th>
<th>TS (MPa)</th>
<th>Ɛ(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1W</td>
<td>5W</td>
<td>1W</td>
</tr>
<tr>
<td>C</td>
<td>420±90\textsuperscript{a1}</td>
<td>330±130\textsuperscript{a1}</td>
<td>19±5\textsuperscript{a1}</td>
</tr>
<tr>
<td>1 %</td>
<td>590±110\textsuperscript{b1}</td>
<td>380±90\textsuperscript{ab2}</td>
<td>23±4\textsuperscript{a1}</td>
</tr>
<tr>
<td>3 %</td>
<td>400±100\textsuperscript{a1}</td>
<td>450±100\textsuperscript{bc1}</td>
<td>19±6\textsuperscript{a1}</td>
</tr>
<tr>
<td>5 %</td>
<td>440±170\textsuperscript{a1}</td>
<td>460±70\textsuperscript{c1}</td>
<td>19±5\textsuperscript{a1}</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b,c} different letter in the same column indicate significant differences among formulations (p<0.05).
\textsuperscript{1,2} different number in the same file indicate significant differences among storage time (p<0.05).
Table 4: The overall migration of control blend films and those containing 1 %, 3 % and 5 % of CNC, in 10% (v/v) ethanol and isoctane food simulants. Mean values ± standard deviation.

<table>
<thead>
<tr>
<th>FILMS</th>
<th>Ethanol 10% (v/v) (mg/kg simulant)</th>
<th>Isoctane (µg/kg simulant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>4.6±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34±3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1%</td>
<td>4.5±0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>22±2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3%</td>
<td>4.8±0.2&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12±3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5%</td>
<td>5.8±1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15±2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ab</sup> different letter in the same column indicate significant differences among formulations (p<0.05).
FIGURE CAPTIONS

Figure 1: FESEM micrographs of the surface of control blend and composite films with different contents of CNC (samples 1 %, 3 % and 5 %).

Figure 2: FESEM micrographs of the cross section of control blend and composite films with CNC contents (samples 1 %, 3 % and 5 %). Higher magnification images from top and bottom of the films are included to observe the different separated phases.

Figure 3: Phase imaging AFM maps of surface of control blend and composite films with different CNC contents (1 %, 3 % and 5 %). Higher magnification for continuous (CP) and dispersed (DP) phases at the film surface.

Figure 4: Maps of Log DTM modulus obtained from AFM in surface of control blend and composite films with different contents of CNC.

Figure 5: FTIR spectra of control blend and composite films with different amounts of CNC (1 %, 3 % and 5 %).

Figure 6: TG (a) and DTG (b) curves obtained from TGA of control blend and composite films with CNCs (1 %, 3 % and 5 %).

Figure 7: Spectral distribution in the UV range of the UV-VIS spectra of control blend (C) and composite films with CNC (1 %, 3 % and 5 %).

Figure 8: Typical strain-stress curves of control blend (C) and composite films with CNCs (1 %, 3 % and 5 %) after 5 weeks of storage.
Figure 1.
Figure 2.
Figure 4.
Figure 5.
Figure 7.
Figure 8.