1 Valorisation of vine shoots for the development
2 of cellulose-based biocomposite films with
3 improved performance and bioactivity

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Graphical Abstract

Abstract

This work reports on the valorization of *Tempranillo* vine shoots for the development of bio-based packaging materials. Cellulose (F3) and nanocellulose (NANO F3) were produced by the conventional method, while less purified cellulosic fractions (F2A) and nanocrystals (NANO F2A) were extracted by simplified protocols (omitting Soxhlet and alkaline treatments) to reduce production costs and environmental impact and evaluate the potential added functionalities of these less purified materials. Although most of the hemicelluloses in F2A were digested upon acid hydrolysis, a small fraction remained in NANO F2A. On the other hand, the presence of a minor xylan fraction in F3 limited the access of sulphuric acid towards the cellulose microfibrils, hindering hydrolysis and producing heterogeneous fibrillar structures in NANO F3. The obtained materials were used to produce cellulosic films, as well as blends with agar, and their performance properties were evaluated. Overall, NANO F2A films showed the best compromise between performance and sustainability and presented additional antioxidant capacity. The properties of the films could be adjusted by the incorporation of agar, improving their ductility and water permeability.

Keywords: cellulosic fractions; bio-based packaging; vine shoots; waste biomass; agar.

1. Introduction

During the last decades, both human and scientific developments have brought the overexploitation of natural resources and led to a massive consumption of fossil fuels, triggering the severe environmental issues that we are currently facing. On the one hand, natural ecosystems have been damaged due to the pollution originated by human and industrial activities. Plastic residues are a particularly hot topic: More than 90% of plastic residues cannot be recycled and they are accumulated in the environment. For instance, up to 95% of the total amount of residues in the Mediterranean Sea are plastic debris [1]. On the other hand, synthetic plastics are obtained from fossil fuels, causing the steady depletion of these non-renewable resources.

In this context, biopolymers (biodegradable polymers and/or obtained from renewable resources) have been explored over the past few years as a sustainable alternative for the replacement of synthetic plastics. Although some biopolymers such as starch and poly(lactic) acid (PLA) are already used in the packaging industry, their commercial grades are often blends with synthetic polymers and the biodegradability of these products is certainly questionable. To make them competitive against conventional plastics, the properties of biopolymers are yet to be improved (especially in terms of mechanical and barrier performance) and their production costs need to be reduced. Furthermore, biopolymers are frequently obtained from food sources such as rice, corn or potato, which is against the circular economy principles. This has motivated the search of alternative and more sustainable sources for the extraction of biopolymers, such as agroindustrial waste and marine biomass. While terrestrial biomass is typically richer in cellulose, marine biomass contains other biopolymers such as agar or alginate [2].

Cellulose is one of the most widely studied biopolymers for the development of bio-based food packaging. Due to its semicrystalline structure, it presents excellent mechanical and barrier properties but limited processability; thus, it is typically used as a reinforcing filler in blends with other biopolymers such as starch [3]. Additionally, when subjected to acid hydrolysis, the amorphous domains of cellulose microfibrils are digested, yielding a highly crystalline material with increased thermal resistance, water and oxygen barrier and stiffness, known as cellulose nanocrystals or nanocellulose. Nanocellulose has been extensively reported in the literature not only as a reinforcing agent in other biopolymers such as starch or PLA [4, 5], but also as film-forming material for food packaging applications [6]. However, as expected, the more the crystalline cellulose is isolated, the lower are the extraction yields. To increase the sustainability of the process, reduce the processing times and increase the yields, alternative protocols which omit several of the cellulose purification steps have been recently reported in the literature for marine waste biomass from *Posidonia oceanica* leaves [6]. The hydrolysis of less purified cellulosic fractions has been shown to produce cellulosic nanocrystals containing hemicelluloses and lipidic compounds, which presented improved mechanical and barrier performance as compared to pure nanocellulose [6]. Due to the very distinct composition of cellulosic biomass depending on its origin, the potential of these alternative protocols is still to be explored when applied to terrestrial sources.

Vine shoots represent an abundant agricultural residue derived from the wine industry [7]. Due to the large costs associated to the management of such an abundant and low-density residue, vine shoots have been traditionally either left in the vineyard to be used as fertilizers or burned. However, burning practices pose severe environmental issues and strict restrictions are applicable in most wine-producing countries [8]. In this context,

strategies for the valorisation of vine shoots, mostly related to biofuel production [7], are currently being sought. Due to the high lignocellulosic content of vine shoots [9], they can also be considered as an exploitable source of cellulose. In fact, some works have reported on the production of pulp and paper [9, 10] and on the extraction of cellulose nanocrystals [11] by the conventional method, although no details on the extraction yields were provided. Moreover, vine shoots have a great potential for the development of bioactive materials, since they are rich in proteins and polyphenols [12] and have been shown to present prebiotic activity [13].

In this work, we investigated the suitability of vine shoots for the extraction of cellulosic fractions and nanocrystals through conventional and simplified protocols. Initially, the composition of vine shoots from two different varieties (*Verdejo* and *Tempranillo*) was characterized to determine the most optimum material for cellulose extraction. The extracted fractions and nanocrystals were subsequently used to produce (nano)cellulosic films and the properties of the obtained materials were evaluated to assess their suitability for food packaging applications. Furthermore, hybrid materials were produced by adding agar into the nanocellulosic films with the aim of reducing their excessively rigid behaviour. Our hypothesis is that simplified purification protocols can yield less purified (nano)cellulosic films from vine shoots waste biomass with similar or even better performance than those obtained using conventional protocols, thus representing a more sustainable way to develop bio-based packaging materials. Moreover, we believe that addition of agar will improve the processability and mechanical properties (in terms of elongation at break) of the typically rigid (nano)cellulosic films.

2. Materials and methods

2.1 Raw materials

Biomass waste material consisting of vine shoots from *Tempranillo* (referred to as TM) and *Verdejo* (referred to as VJ) varieties was kindly provided by Matarromera, Valladolid (Spain) in February 2019. Vine shoots were initially converted into smaller pieces in order to facilitate their further processing by an industrial crusher. The resulting material was milled and sieved manually until obtaining a homogeneous powder (0.5 mm). Commercial agar PRONAGAR (batch reference H-3544/19) was supplied by Hispanagar (Burgos, Spain).

2.2 Compositional analysis of vine shoots

2.2.1 Lignin content

The Klason lignin content was determined according to the TAPPI T222 om-06 method. Briefly, 3 mL of 72% H₂SO₄ (v/v) were vigorously mixed with 300 mg of dry biomass (from both TM and VJ varieties) in glass tubes. The tubes were then placed in a water bath at 30 °C for 1 hour and vortexed every 10 minutes. After that, 84 mL of distilled water were added to each tube and mixed. The resulting material was autoclaved for 1 hour at 121 °C and then cooled down with ice until reaching room temperature. The content of the tubes was subsequently filtered, and the solid material was dried in an oven at 105 °C overnight. The lignin content was calculated gravimetrically. The determinations were carried out in triplicate.

2.2.2 Holocellulose content

The holocellulose content was determined according to the ASTM D1104-56 method. Briefly, 1 g of dry biomass was added to 150 mL of distilled water, pre-heated at 70 °C. While stirring, 1 g of NaClO₂ and 0.2 mL of acetic acid were added every 1 hour. This

process was repeated three times, accounting for a total time of 4 hours. The obtained material was then placed in an ice-bath to stop the reaction. After several washing cycles with distilled water (until obtaining a clear filtrate), the material was dried in an oven at 105 °C overnight. The holocellulose content was calculated gravimetrically. The determinations were carried out in triplicate.

2.2.3 Ash content

The ash content was determined by dry biomass calcination, according to the standard TAPPI T211 om-07 method [14]. Briefly, dry biomass samples were placed in a muffle at 525 °C for at least 4 hours. The ash content was gravimetrically determined after combustion. The determinations were carried out in duplicate.

2.2.4 Lipid content

The lipid content was estimated by the Soxhlet extraction method. Approximately 9 g of dry biomass were placed in a Dumas filter and treated with 800 mL of a 2:1 toluene:ethanol mixture overnight. The lipid content was calculated gravimetrically after drying the extracted solid fraction. The determinations were carried out at least in triplicate.

2.2.5 Protein content

Samples were analyzed for total nitrogen content using an Elemental Analyser Rapid N Exceed (Paralab S.L., Spain). Approximately 100 mg of dry biomass were pressed into pellets and then analyzed using the Dumas method, which is based on the combustion of the sample and subsequent detection of the released N₂ [15]. The protein content was

calculated from the nitrogen content multiplied by a factor of 6.25. The determinations were carried out in triplicate.

2.2.6 Total phenolic content

The total phenolic content of the vine shoots was estimated by the Folin-Ciocalteu colorimetric assay [16]. Briefly, the Folin-Ciocalteu reagent was diluted 1:10 (v/v) with distilled water and 1 mL of the final dilution was mixed with 0.2 mL of the sample (aqueous dispersions of the biomass at 20 mg/mL) at room temperature. Finally, 0.8 mL of sodium carbonate (75 mg/mL) were added and the samples were heated up to 50 °C for 30 minutes. Absorbance values were read at 750 nm wavelength. The determinations were carried out in triplicate.

2.3 Preparation of cellulosic fractions and nanocrystals

Two purification procedures described in a previous work [6] were carried out to sequentially remove cell wall components from the raw vine shoots and obtain pure or partially pure cellulose. The conventional process consisted of an initial Soxhlet extraction to remove pigments and lipids, followed by a treatment with NaClO₂ to remove lignin and a final alkaline treatment with KOH to remove the hemicelluloses, yielding more purified cellulose (labelled as F3). The alternative protocol omitting the Soxhlet and KOH treatments was also applied, obtaining a less purified cellulosic fraction (referred to as F2A). Both fractions (F3 and F2A) were obtained as partially hydrated gel-like materials, which were stored in the fridge until further use.

Both cellulosic fractions were used as the starting materials to produce cellulosic nanocrystals by means of acid hydrolysis. An optimized method, previously applied for

the extraction of cellulose nanocrystals from *Posidonia oceanica* waste biomass [6], with some minor modifications, was applied. Briefly, the gel-like cellulosic fractions were immersed in a hot (50 °C) H₂SO₄ solution (30% w/w), with a ratio of 1.5 g fraction (in dry basis)/100 mL H₂SO₄ and kept under stirring for 1 hour. Additionally, the F3 fraction was subjected to a longer hydrolysis of 5 hours to evaluate the effect of hydrolysis time on the properties of the nanocrystals. After the hydrolysis, the acid slurries were subjected to several centrifugation and washing cycles to remove the acid and the pH was adjusted to 7 with diluted NaOH. The obtained nanocrystals (labelled as NANO F2A, NANO F3 and NANO F3 5h) were stored in the fridge as partially hydrated gel-like materials, until further use.

2.4 Production of pure (nano)cellulosic films and hybrid films with agar

Pure cellulosic films were produced by adding 0.25 g of cellulosic materials to 50 mL of distilled water and dispersing them by ultra-turrax homogenization followed by mild sonication until obtaining homogeneous suspensions. These suspensions were then vacuum filtered using PTFE filters with 0.2 µm pore size to remove water. The solid material remaining in the filter was subsequently dried at room temperature overnight (ca. 20 °C, 40% RH). The obtained films were named as F2A, F3, NANO F2A, NANO F3 and NANO F3 5h.

Additionally, composite films were prepared using commercial agar in proportions of 20%, 40% and 80% with respect to the total amount of solids (fixed at 0.25 g). For this aim, the proportional weight of agar was dissolved in the corresponding water volume (10 mL, 20 mL and 40 mL) at 50 °C while the nanocellulosic materials (NANO F2A and NANO F3) were dissolved in the remaining water volume until reaching 50 mL. The

obtained suspensions followed the same protocol as the one followed for pure cellulosic films. The resulting films were named as NANO F2A 20% AGAR, NANO F2A 40% AGAR, NANO F2A 80% AGAR, NANO F3 20% AGAR, NANO F3 40% AGAR, NANO F3 80% AGAR.

All obtained films were stored in equilibrated relative humidity cabinets at 53% RH and 25 °C for three days prior to their characterization.

2.5 Carbohydrate composition of the cellulosic fractions and nanocrystals

The carbohydrate content and sugar composition of the cellulosic fractions and nanocrystals was determined after sulphuric hydrolysis, as previously described [17]. The monosaccharides were analysed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) with a ICS-3000 (Dionex) equipped with a Carbowac PA 1 column (4 × 250 mm, 10 µm, Dionex). Control samples of known concentrations of mixtures of glucose, fucose, galactose, arabinose, xylose, mannose, galacturonic acid and glucuronic acid were used for calibration. All experiments were carried out in triplicate.

2.6 Attenuated total reflectance (ATR) FT-IR analysis

Freeze-dried fractions and nanocrystals were analysed by FT-IR in attenuated total reflectance (ATR) mode using a Thermo Nicolet Nexus (GMI, USA) equipment. The spectra were taken at 4 cm⁻¹ resolution in a wavelength range between 400-4000 cm⁻¹ and averaging a minimum of 32 scans.

2.7 Thermogravimetric analyses (TGA)

Thermogravimetric curves (TG) were recorded with a Setaram Setsys 16/18 (SETARAM Instrumentation, France). The samples (ca. 10 mg of the cellulosic fractions and nanocrystals) were heated from 30 to 1000°C with a heating rate of 10°C/min under nitrogen atmosphere. Derivative TG curves (DTG) express the weight loss rate as a function of temperature.

2.8 Transmission electron microscopy (TEM)

One drop (8 µL) of a 0.001% aqueous suspension of the different cellulosic nanocrystals obtained was allowed to dry on a carbon coated grid (200 mesh). The nanocrystals were stained with uranyl acetate. TEM was performed using a JEOL 1010 at an accelerating voltage of 80 kV. The size of the nanocrystals was determined by image analysis using the ImageJ-win64 software. At least 5 different images were analysed and the results were expressed as mean ± standard deviation.

2.9 Scanning electron microscopy (SEM)

SEM was conducted on a Hitachi microscope (Hitachi S-4800) at an accelerating voltage of 10 kV and a working distance of 8-16 mm. Small samples (~5 mm² area) of the (nano)cellulosic films and their blends with agar were cut to observe their surface. The samples were then sputtered with a gold–palladium mixture under vacuum during 3 minutes before their morphology was examined.

2.10 X-ray diffraction (XRD)

XRD measurements of the pure (nano)cellulosic films and their blends with agar were carried out on a D5005 Bruker diffractometer. The instrument was equipped with a Cu tube and a secondary monochromator. The configuration of the equipment was θ –2 θ , and

the samples were examined over the angular range between 3°–60° with a step size of 0.02° and a count time of 200 s per step. Peak fitting was carried out by using the Igor software package (Wavemetrics, Lake Oswego, Oregon) as described in a previous work [18]. The crystallinity index X_C was determined from the obtained fitting results by applying the following equation:

$$X_C(\%) = \frac{\sum A_{Crystal}}{A_{Total}} \times 100 \quad (1)$$

where A_{Total} is the sum of the areas under all the diffraction peaks and $\sum A_{Crystal}$ is the sum of the areas corresponding to the three crystalline peaks from cellulose I.

2.11 Mechanical properties

Tensile tests were carried out at ambient conditions on a Mecmesin MultiTest 1-i (1 kN) machine (Virginia, USA) with the EmperorTM software, according to ASTM standard method D882-09 [19]. Pre-conditioned rectangular-shaped specimens with initial gauge length of 8 cm and 1 cm in width were cut directly from the films. A fixed crosshead rate of 10 mm/min was utilized in all cases. The elastic modulus (E), tensile strength (TS), and elongation at break (EAB) were determined from the stress-strain curves, estimated from force–distance data obtained for the different films. At least, five specimens of each film were tensile tested to obtain statistically meaningful results.

2.12 Water vapor permeability (WVP)

Direct permeability to water was determined from the slope of the weight gain versus time curves at 25 °C. The films were sandwiched between the aluminum top (open O-ring) and bottom (deposit for the silica) parts of a specifically designed permeability cell with screws. A Viton rubber O-ring was placed between the film and bottom part of the cell to enhance sealability. These permeability cells containing silica were then placed in

an equilibrated relative humidity cabinet at 75% RH and 25 °C. The weight gain through a film area of 0.001 m² was monitored and plotted as a function of time. Cells with aluminum films (with thickness of ca. 11 µm) were used as control samples to estimate the weight gain through the sealing. The tests were done at least in triplicate.

2.13 Contact angle measurements

Contact angle measurements were carried out at ambient conditions in a Video-Based Contact Angle Meter model OCA 20 (DataPhysics Instruments GmbH, Filderstadt, Germany). Contact angle values were obtained by analyzing the shape of a distilled water drop after it had been placed over the film for 15 s. Image analyses were carried out by SCA20 software.

2.14 Optical properties

The transparency of the films was determined through the surface reflectance spectra in a spectrophotometer CM-3600d (Minolta Co., Tokyo, Japan) with a 10 mm² illuminated sample area. Measurements were taken in duplicate for each sample using both a white and a black background.

Film transparency was evaluated through the internal transmittance (T_i) (0-1, theoretical range) by applying the Kubelka-Munk theory for multiple scattering to the reflection data. Internal transmittance (T_i) of the films was quantified using Eq. (2). In this equation, R_0 is the reflectance of the film on an ideal black background. Parameters a and b were calculated by Eqs. (3) and (4), where R is the reflectance of the sample layer backed by a known reflectance R_g .

$$T_i = \sqrt{(a - R_0)^2 - b^2} \quad (2)$$

$$a = \frac{1}{2} \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \quad (3)$$

$$b = (a^2 - 1)^{\frac{1}{2}} \quad (4)$$

329

330 **2.15 β -Carotene-linoleic acid assay**

331 The antioxidant capacity of the (nano)cellulosic fractions was also evaluated by the β -
 332 carotene-linoleic acid assay [20]. In brief, 4 mg of β -carotene were dissolved in 20 mL of
 333 chloroform. 2 mL of this solution were placed on a rotary evaporator and the chloroform
 334 was evaporated. Then, 50 μ L of linoleic acid and 400 mg of Tween 40 were added and
 335 the content of the flask was mixed with stirring. After that, 100 mL of aerated distilled
 336 water was transferred to the flask and stirred vigorously. 5 mL of the prepared β -carotene
 337 emulsion were transferred to a series of tubes containing a fixed weight of 10 mg of each
 338 (nano)cellulosic fraction in film-form, or 0.5 mL of BHT (0.1-1 mg/mL) (as a positive
 339 control) and 0.5 mL of distilled water (as the negative control). The samples were
 340 incubated in a water bath at 50 °C for 120 min. The absorbance of each fraction at 470
 341 nm was measured every 30 minutes using a spectrophotometer. All the determinations
 342 were carried out in triplicate.

343

344 **2.16 Statistical analysis**

345 Analysis of variance (ANOVA) followed by a Tukey-b test were used when comparing
 346 more than two data sets, after confirming the homogeneity of variances by the Levene
 347 test using IBM SPSS Statistics software v.26. All data have been represented as the
 348 average $\pm$ standard deviation. Significant differences ($p \leq 0.05$) are denoted by showing
 349 the data provided in tables with different letters.

350

3. Results and Discussion

3.1. Compositional characterization of vine shoots

In the first stage of this work, the composition of vine shoots from two different varieties (TM and VJ) was characterized to determine the most optimum raw material for the extraction of cellulose. The results, shown in Table 1, evidenced no significant differences between varieties, except for the higher lipid content in VJ. This is in agreement with a previous work in which very similar composition was reported for four different vine shoot varieties [10]. Holocellulose represented the major component in both varieties, with contents of ca. 71% for VJ and 72% for TM. As expected, lignin was the second major component, representing ca. 22-23% of the biomass, in line with previous studies [10]. Only minor amounts of lipids (ca. 2-7%), proteins (ca. 4-5%) and ashes (ca. 2-4%) were detected in both varieties. Additionally, no phenolic compounds could be quantified using the Folin-Ciocalteu method. This does not necessarily imply a low phenolic content in the raw biomass, since, in fact, vine shoots have been proposed as a good source for the extraction of polyphenols [12, 21]. Instead, this suggests that the existing phenolic compounds are bound to the lignocellulosic structure in the intact cell walls composing the vine shoot tissues. Overall, our results, together with the compositional data previously reported in the literature for other varieties [10, 22, 23], demonstrate that the composition of vine shoots is quite reproducible along different varieties.

Table 1. Composition of the raw biomass from *Tempranillo* (TM) and *Verdejo* (VJ) vine shoots. Values in the same column followed by different letters are significantly different ($p \leq 0.05$).

Lignin	Holocellulose	Ash	Lipids	Proteins
(%)	(%)	(%)	(%)	(%)

TM	22.4 ± 2.4^a	72.0 ± 4.4^a	2.3 ± 0.3^a	6.7 ± 2.1^a	5.3 ± 0.3^a
VJ	23.1 ± 2.4^a	70.7 ± 2.4^a	3.6 ± 0.4^a	2.4 ± 1.3^b	4.0 ± 0.4^a

374

375 FT-IR characterization was also carried out to further investigate any possible minor
376 compositional differences. As shown in Figure 1A, both vine shoot varieties showed very
377 similar spectra. The broad bands at $3600\text{--}3200\text{ cm}^{-1}$ and $3000\text{--}2900\text{ cm}^{-1}$, characteristic
378 from O—H and C—H stretching, respectively [24], presented very similar shapes.
379 Moreover, the band located at 1730 cm^{-1} , corresponding to the C=O stretching vibration
380 of ester groups from lignin and hemicelluloses [25], was also present in both varieties.
381 Interestingly, the band at 1040 cm^{-1} , which is usually related to xylans [24, 26], was also
382 visible. Finally, the presence of cellulose in both varieties was confirmed by the
383 appearance of several characteristic bands, such as those located at 1420, 1300 and 1100
384 cm^{-1} [24, 27].

385

386 The thermal stability of both vine shoot varieties was also assessed by TGA analyses and
387 the results are shown in Figure 1B. As observed, both varieties showed a very similar
388 behavior, typical from multicomponent materials, displaying a three-step degradation
389 profile. The most intense peak was the one with its maximum at $\sim 330\text{ }^{\circ}\text{C}$, which
390 corresponds to the thermal degradation of cellulose [25]. This peak was slightly
391 overlapped with a less intense peak with its maximum around $260\text{ }^{\circ}\text{C}$, which is typically
392 attributed to the degradation of hemicelluloses [25]. Overall, both vine shoot varieties
393 presented almost identical thermal stability, which is not surprising given their
394 compositional similarity (cf. Table 1).

395

Figure 1. (A) FT-IR spectra of both vine shoots varieties (*Tempranillo* (TM) and *Verdejo* (VJ)). TM spectrum has been offset for clarity. Arrows are pointing towards the bands characteristic from lignin and hemicelluloses such as xylans (thick arrows), and cellulosic crystalline peaks (thin arrows). (B) Derivative thermogravimetric (DTG) curves of TM and VJ vine shoots.

Given the compositional similarity between both vine shoot varieties and, in particular, their comparable holocellulosic content, TM was chosen as the raw material to extract

cellulosic fractions and nanocrystals. The choice was done on the basis of the greater availability of TM vine shoots as compared with VJ, since the former is much more cultivated around the world (and at a local/regional level) and thus represents a more profitable market opportunity [28].

3.2. Production and characterization of cellulosic fractions and nanocrystals

TM vine shoots were subjected to the purification protocols described in section 2.3 to produce cellulosic fractions and nanocrystals with different degrees of purity. As expected, the simplified protocol produced less purified fractions and nanocrystals with higher yields (ca. $63 \pm 3\%$ for F2A and $27 \pm 3\%$ for NANO F2A) than the standard cellulose purification method (ca. $22 \pm 2\%$ for F3 and $14 \pm 1\%$ for NANO F3). On the other hand, the application of the acid hydrolysis reduced the extraction yields, being this decrease more evident in the case of the F2A fraction. This is reasonable taking into account that amorphous hemicelluloses were expected to remain in F2A, while F3 was expected to be almost pure cellulose. The obtained extraction yields are consistent with those previously reported for the fractions (60% for F2A and 25% for F3) and nanocrystals (26% for NANO F2A and 14% for NANO F3) extracted from *Posidonia oceanica* waste biomass by applying the same protocols [6]. The overall initial composition of *Posidonia* was not substantially different to that from vine shoots (13% ash, 18% lignin and 59% holocellulose) [6] and, therefore, similar extraction yields were expected for both biomass sources.

XRD analyses were also carried out to study the crystalline structure of cellulose in the fractions and nanocrystals and the obtained patterns are shown in Figure 2. As observed, all the samples were characterized by the appearance of a broad diffraction band at 12–

18° (which is actually composed of two overlapped peaks located at 15.0°, 16.6°), followed by a sharper peak located at ca. 22.6°, indicating the presence of crystalline cellulose I [29]. Additionally, a small peak, located at 27 °, most likely arising from the presence of mineral compounds [30] was visible in F2A. Figure 2 clearly shows that the intensity of the cellulose crystalline peaks significantly increased when subjecting the less purified F2A fraction to the acid hydrolysis, while minor differences were seen between the purified cellulose fraction F3 and the extracted nanocrystals. In fact, cellulose crystallinity, which was calculated by estimating the area of the three crystalline peaks, markedly increased for F2A after the hydrolysis treatment (from ca. 43% for F2A to 85% for NANO F2A), while it remained almost constant for F3 (ca. 85% for F3 to 89% for NANO F3). The increased crystallinity of F3 as compared to F2A can be explained by the removal of a significant amount of amorphous material, such as hemicelluloses, by the conventional purification protocol applied. Similarly, when subjecting F2A to the acid hydrolysis, the remaining hemicelluloses and other components such as lipids are expected to be easily digested. However, the very minor effect of the acid hydrolysis on the cellulose crystallinity was surprising and it was against the crystallinity increase previously reported for the fractions and nanocrystals extracted from *Posidonia oceanica* biomass using the same purification protocol (from ca. 67% for F3 to 77% for NANO F3) [6]. However, it should be noted that the cellulosic fraction F3 extracted from *Posidonia oceanica* presented a less crystalline structure than that of the cellulose from vine shoots, hence making the former more susceptible to acid digestion. To evaluate whether the hydrolysis time had been insufficient to digest the cellulose amorphous domains in F3, a longer hydrolysis time of 5h was also tested. The yield for the extracted nanocrystals ($13.8\% \pm 1.1\%$) was similar to the conventional shorter hydrolysis, while the crystallinity index (ca. 87%) was seen to slightly decrease. This is consistent with previous studies, as

longer hydrolysis times might decrease crystallinity values after the optimum time is reached [31]. As a result, the hydrolysis parameters need to be adjusted to each biomass source as applying excessive acid concentrations or too long hydrolysis times can also lead to partial degradation of the cellulose crystalline structure [31]. In the case of the TM vine shoots, it seems that the purified cellulose presented a quite crystalline structure, with a minor fraction of amorphous cellulose which was not accessible to the sulphuric acid and thus, remained non-digested even when extending the hydrolysis time.

Table 2. Extraction yield and crystallinity index (Xc) of the different cellulosic fractions (F2A and F3) and nanocrystals (NANO F2A and NANO F3) extracted from *Tempranillo* (TM) vine shoots.

	Yield (%)	Xc (%)
F2A	62.5 ± 1.8	43.3
F3	21.7 ± 2.5	85.4
NANO F2A	27.2 ± 0.4	84.9
NANO F3	14.2 ± 2.7	89.3

Figure 2. XRD patterns from the unpurified cellulosic fractions and nanocrystals (F2A and NANO F2A) (A) and pure cellulose and nanocrystals (F3 and NANO F3) (B) extracted from *Tempranillo* (TM) vine shoots.

The FT-IR spectra from the cellulosic fractions and nanocrystals extracted from TM vine shoots are shown in Figure 3A. The peak located at 1730 cm^{-1} , characteristic from the acetyl groups from hemicelluloses and/or lignin [24], was visible in F2A (as expected, since the simplified extraction was not aimed to remove all the hemicelluloses present in the raw biomass). Interestingly, the peak remained visible, although with lower intensity, in NANO F2A, suggesting that a certain fraction of the hemicelluloses present in F2A was resistant to the hydrolysis. This peak was not visible in F3, suggesting that, if hemicelluloses remained in the material, they represented only a very minor fraction. Moreover, the region where most of the cellulose characteristic bands are located ($\sim 1200\text{--}800\text{ cm}^{-1}$ [24, 27]) was affected depending on the applied treatment. As expected, the peaks within this region became sharper and more intense after hydrolysis of the less purified fraction (F2A). However, in the case of the purified cellulose (F3), the intensities of these bands did not increase (or were even slightly decreased) after the hydrolysis. This

suggests, in line with the XRD results, that while the sulphuric acid was able to digest most of the amorphous hemicelluloses initially present in F2A, it was not able to penetrate the structure of cellulose microfibrils in F3 to digest the remaining amorphous domains. It should also be noted that the band located at 768 cm^{-1} (pointed out with arrows in Figure 3A) was detected in NANO F2A and NANO F3, suggesting the presence of sulphate groups as a result of the sulphuric acid hydrolysis [27].

TGA analyses of the cellulosic fractions and nanocrystals were also performed to assess their thermal stability and the results are shown in Figure 3B. As expected, F3 and NANO F3 only showed one sharp and defined peak with its maximum at around $334\text{ }^{\circ}\text{C}$, characteristic from the thermal degradation of cellulose [25]. On the other hand, F2A showed the same additional peak detected in the raw vine shoots at around $260\text{ }^{\circ}\text{C}$ (cf. Figure 1B), ascribed to the degradation of hemicelluloses. This peak became less intense after acid hydrolysis, but it could still be detected in NANO F2A, confirming that although most of the amorphous hemicelluloses were digested by the sulphuric acid, a small fraction of more resistant hemicelluloses was not hydrolyzed. It should be noted that in the case of NANO F2A, the cellulosic degradation peak was slightly shifted towards lower temperatures. This could be ascribed to the presence of residual sulphate groups in the surface of the extracted nanocrystals, which are known to decrease the thermal resistance of cellulose [32].

Figure 3. (A) FT-IR spectra of the cellulosic fractions (F2A and F3) and nanocrystals (NANO F2A and NANO F3) extracted from *Tempranillo* (TM) vine shoots. Arrows are pointing towards characteristic bands in the spectra. (B) derivative thermogravimetric (DTG) curves of the cellulosic fractions and nanocrystals extracted from TM vineshoots.

Sulphuric acid treatment of cellulosic materials has been shown to proceed with a preferential digestion of amorphous domains in the longitudinal axis of cellulose microfibrils. Thus, longer or more aggressive hydrolysis conditions have a strong impact

on the aspect ratio (length/width) of the extracted nanocrystals [32]. To evaluate the morphology of the cellulosic nanocrystals extracted from vine shoots, they were characterized by TEM and representative images are shown in Figure 4. It could be seen that NANO F2A (Fig. 4A) was composed of long fibrillar structures ($847 \pm 51 \mu\text{m}$, with an aspect ratio of 43.4) with a significant degree of agglomeration. This is reasonable considering that, as suggested by FT-IR and TGA analyses, a significant amount of hemicelluloses remained in NANO F2A, since hemicelluloses are known to act as a cross-linking agents in plant cell walls [6]. In the case of NANO F3, more heterogeneous structures could be visualized. Most of the material corresponded to isolated long fibrils ($604 \pm 48 \mu\text{m}$, with an aspect ratio of 31.9) (Fig. 4B). However, additional structures with much shorter lengths ($208 \pm 12 \mu\text{m}$, with an aspect ratio of 13.5), which may correspond to hydrolyzed cellulose fragments, could be also detected (Fig. 4C). These results suggest that the sulphuric acid hydrolysis proceeded heterogeneously, with most of the cellulose microfibrils remaining intact, while the hydrolysis of more exposed cellulose regions produced a minor fraction of shorter needle-like structures.

Figure 4. TEM images of the cellulosic nanocrystals extracted from *Tempranillo* (TM) vine shoots. (A) NANO F2A (unpurified nanocrystals) and (B and C) NANO F3 (pure cellulose nanocrystals).

The obtained results seemed to point towards two interesting hypotheses: (i) a certain fraction of hemicelluloses was resistant to the sulphuric acid hydrolysis and remained in the less purified NANO F2A nanocrystals and (ii) the access of sulphuric acid towards the cellulose microfibrils in F3 was somehow obstructed; as a result, the hydrolysis was not efficient and proceeded heterogeneously. To investigate the impact of the different extraction protocols on the carbohydrate composition of the fractions and nanocrystals extracted from TM vine shoots, monosaccharide analyses were carried out and the results are displayed in Table 3. As expected, glucose was the major component in all fractions and could be fully ascribed to cellulose, as small quantities of mixed linkage β -glucan (previously reported as <5% of the polysaccharide fraction in the raw material) [33], may have been probably removed in the treated fractions. As in hardwoods, xylan in *Vitis vinifera* has been reported to consist of an acetylated glucuronoxylan with a backbone of β -(1 $\rightarrow$ 4)-linked xylopyranosyl units with α -(1 $\rightarrow$ 2)-linked 4-O-methyl- α -glucopyranosyl residues (MeGlcA). It is well known that xylan is the main hemicellulose in most flowering plants, including vine shoots, playing a crucial role in their cell wall

architecture by its direct interaction with cellulose and lignin [34]. Previous works have demonstrated that even minor amounts of xylans in the secondary cell walls from higher plants have a strong impact on the structure of cellulose microfibrils [35].

Recent studies have elucidated that an even pattern of substitution of MeGlcA or acetyl moieties in xylan allows for the xylan chains to be docked onto the hydrophobic or hydrophilic surfaces, respectively, of cellulose microfibrils, adopting a preferential two-fold screw conformation [36-38]. This tight interaction of xylan with cellulose is partly responsible for the inherent recalcitrance of lignocellulosic biomass and might explain a xylan fraction remaining in all samples [39, 40]. This is mostly patent in the less aggressively treated F2A, and slightly decreasing xylan contents are noted with increasing aggressiveness of the applied treatments. Interestingly, a remarkable xylose fraction was present in NANO F2A, seemingly resistant to the sulphuric hydrolysis (~12 %). This xylan may have prevented the sulphuric acid to reach the amorphous cellulosic domains and thus reduced its effectiveness. Previous reports have also noted an abnormal resistance of vine stalks to kraft pulping, compared to hardwood, which was attributed to a different lignin structure [41].

Table 3. Monosaccharide relative content (%) of the cellulosic fractions (F2A and F3) and nanocrystals (NANO F2A and NANO F3) extracted from *Tempranillo* (TM) vine shoots. Values in the same line followed by different letters are significantly different ($p \leq 0.05$).

%*	F2A	F3	NANO F2A	NANO F3
Arabinose	1.48 ± 0.25 ^a	0.06 ± 0.03 ^b	0.06 ± 0.01 ^b	0.01 ± 0.01 ^b
Galactose	1.89 ± 0.97 ^a	0.46 ± 0.03 ^b	0.19 ± 0.05 ^c	0.05 ± 0.02 ^c

Glucose	71.18 ± 1.24^a	85.99 ± 0.48^b	90.75 ± 0.32^c	93.54 ± 0.23^d
Xylose	20.24 ± 0.30^a	10.64 ± 0.55^b	7.98 ± 0.52^c	5.66 ± 0.45^d
GalA	3.27 ± 0.06^a	2.20 ± 0.09^b	0.50 ± 0.15^c	0.29 ± 0.04^c

*The contents of fucose, rhamnose, mannose and 4-O-methyl-glucuronic acid was below 1% in all fractions.

3.3. Characterization of pure (nano)cellulosic films and their blends with agar

The extracted cellulosic fractions and nanocrystals were subsequently used to produce biopolymeric films and their performance properties were characterized to evaluate their potential to be used as food packaging materials. Since one of the main issues of cellulosic films is their excessive rigidity [6, 42], agar was incorporated into the nanocellulosic films at different concentrations to evaluate its compatibility with cellulose and the effect on the performance of the hybrid films. Agar was chosen as the additive for these films since previous works have reported on the more ductile behavior of this biopolymer as compared with cellulose [43, 44].

The morphology of the produced films was evaluated by SEM and representative images are shown in Figure 5. As expected, rougher surfaces with larger fibrillar structures could be observed in the less purified materials (F2A and NANO F2A), while smoother surfaces were seen in F3 and NANO F3. There was also a clear tendency of more homogeneous and smoother film surfaces after the acid hydrolysis in the more purified F3 materials. However, F2A fraction showed a more homogeneous surface in comparison with NANO F2A that hindered the presence of larger hemicellulosic structures (inset in Fig. 5A). Interestingly, the addition of agar, even at the lowest content (20% w/w), had a strong impact on the surface morphology of the films. In general, more continuous surfaces,

where the cellulosic fibrillar structures seemed to be embedded in an amorphous agar coating layer, could be observed. A similar effect has been previously reported for the residual agar remaining in less purified cellulosic films from red seaweed residues [30].

Figure 5. SEM images of the surface from the pure (nano)cellulosic films obtained from *Tempranillo* (TM) vine shoots (A) F2A (unpurified fraction), (B) F3 (pure cellulose), (C) NANO F2A (unpurified nanocrystals), (D) NANO F3 (cellulose nanocrystals) and of their blends with agar (E) NANO F2A+20%AGAR, (F) NANO F3+20%AGAR, (G) NANO F2A+40%AGAR, (H) NANO F3+40%AGAR, (I) NANO F2A+80%AGAR and (J) NANO F3+80%AGAR. Insets represent regions of the films at higher magnification (scale bars represent 100 μ m).

The visual aspect and transparency of the films were also evaluated. As observed in Figure S1A, the larger size of the fibrillar structures composing the films from the cellulosic fractions produced more opaque materials, while the transparency was dramatically improved with the application of the acid hydrolysis, especially in NANO F2A due to the removal of amorphous hemicelluloses and the production of more homogeneous materials with smaller fibrils. Moreover, the presence of non-cellulosic components produced a yellowish tonality in the F2A and NANO F2A films. The hybrid films containing agar were found to be more transparent than the pure (nano)cellulosic films, which may be explained by the amorphous structure of agar [45]. The internal transmittance values of the films, shown in Figures S1B and S1C, confirmed that the acid hydrolysis treatment produced a marked increase in the transparency of the films.

Furthermore, while the blends with agar displayed higher transparency values than the pure (nano)cellulosic films, the amount of added agar did not show any significant effect.

The performance of the films was evaluated in terms of mechanical and water barrier properties and the obtained results are summarized in Table 4. With regards to the mechanical properties, the films from the different (nano)cellulosic fractions showed a clearly distinct behavior. The less purified fraction (F2A) presented very poor mechanical performance; however, after the short hydrolysis treatment the properties of the material (NANO F2A) were significantly improved, especially in terms of rigidity and strength (as suggested by the elastic modulus and tensile strength values). Such a dramatic improvement can be attributed to the digestion of most of the amorphous hemicelluloses initially present in F2A, giving rise to more crystalline materials, with a much homogeneous structure. Indeed, the purification of cellulose by applying the conventional protocol had a more marked positive effect and F3 presented the best compromise between strength and ductility. Surprisingly, the acid hydrolysis of F3 was detrimental for the mechanical performance of the films, reducing the elastic modulus, tensile strength and elongation at break of NANO F3. This supports the hypothesis that most of the cellulose microfibrils in F3 were not accessible to the sulphuric acid and only a small fraction of more accessible cellulose was digested. As a result, heterogeneous nanofibrillar structures with very different sizes, as shown by TEM (cf. Figure 4), were produced. The presence of shorter needle-like structures, with lower aspect ratios, may have been detrimental for the mechanical properties of NANO F3. In fact, the films from the cellulosic nanocrystals extracted by applying a longer hydrolysis of 5h showed even worse mechanical performance than NANO F3, presenting similar elastic modulus (6.5 ± 0.5 GPa) but remarkably lower tensile strength (27.8 ± 1.7 MPa) and elongation ($0.8 \pm$

0.1 %). This is in line with the reduced extraction yield and crystallinity determined for these nanocrystals and confirms that achieving a complete hydrolysis of the amorphous cellulose was not a matter of time. It seems that the small fraction of tightly bound xylan remaining in F3 hindered the access of the sulphuric acid towards certain regions of the cellulose microfibrils.

As already anticipated, the (nano)cellulosic films presented a very rigid behavior, with high elastic moduli but low elongation values. As deduced from the results shown in Table 4, the addition of agar into the nanocellulosic films had a plasticizing effect (reducing the elastic modulus, while increasing the elongation). This effect was more evident with increasing agar contents, reaching a ~3-fold increase in the elongation at break with 80% agar. On the other hand, tensile strength was significantly improved at higher agar contents (40% and 80%). It should be noted that the higher the agar content in the hybrid films, the more their mechanical properties resembled those of pure agar films [43]. Previous works have reported on the opposite effect of adding moderate loadings (1-20% w/w) of cellulosic additives (such as commercial microcrystalline cellulose and nanocrystals) to agar-based films produced by casting, i.e. cellulose acted as a plasticizing agent with slight increase on the stiffness of the composites [46, 47]. Thus, the mechanical properties of the hybrid films can be adjusted depending on their intended application by selecting the optimum content of (nano)cellulosic fractions and agar. It is to be highlighted that, in general, the (nano)cellulosic films and their blends with agar presented similar or even superior mechanical performance than commercial biopolymers such as thermoplastic corn starch (TPCS) or PLA (cf. Table 4). In parallel, similar materials obtained from *Posidonia oceanica* waste biomass showed increased rigidity (E and TS) with similar or lower EAB, demonstrating the effectiveness of

applying these kinds of protocols to both terrestrial and marine resources [6]. However, it should be noted that the EAB values of the hybrid films were still lower than those reported for commercial TPCS and PLA. In this sense, the obtained materials might be more suitable for rigid packaging structures, where high ductility attributes are not required. Furthermore, increasing further the agar content or exploring the production of blends with different hydrocolloids could also help obtaining more ductile materials.

Table 4. Mechanical properties, water vapor permeability and contact angle of the pure (nano)cellulosic films obtained from *Tempranillo* (TM) vine shoots and of their blends with agar.

E: elastic modulus; TS: tensile strength; EAB: elongation at break; WVP: water vapor permeability. Values in the same column followed by different letters are significantly different ($p \leq 0.05$).

n.m.: not measured.

	E (GPa)	TS (MPa)	EAB (%)	WVP (Kg·m/s·m ² ·Pa) ·10 ⁻¹⁴	Contact Angle (°)
F2A	0.2 ± 0.0 ^e	0.8 ± 0.1 ^e	0.5 ± 0.1 ^d	58 ± 0.2 ^c	n.m.
NANO F2A	5.5 ± 0.2 ^b	42.1 ± 2.0 ^c	1.1 ± 0.1 ^c	5.9 ± 0.2 ^a	48.5 ± 5.1 ^c
NANO F2A+20% AGAR	4.5 ± 0.4 ^c	44.6 ± 2.4 ^c	1.6 ± 0.1 ^c	5.6 ± 0.3 ^a	105.9 ± 10.2 ^a
NANO F2A+40% AGAR	4.2 ± 0.4 ^c	50.8 ± 2.7 ^b	2.2 ± 0.2 ^b	6.2 ± 0.1 ^a	99.3 ± 5.8 ^a
NANO F2A+80% AGAR	3.3 ± 0.3 ^d	56.8 ± 5.1 ^{ab}	3.1 ± 0.3 ^a	8.1 ± 0.2 ^b	95.3 ± 8.0 ^a
F3	7.8 ± 0.7 ^a	67.1 ± 4.9 ^a	2.1 ± 0.2 ^b	8.5 ± 0.2 ^b	n.m.
NANO F3	5.9 ± 0.8 ^b	47.2 ± 4.2 ^{bc}	1.2 ± 0.1 ^c	4.9 ± 0.2 ^a	67.9 ± 10.2 ^b
NANO F3+20% AGAR	4.9 ± 0.3 ^{bc}	44.8 ± 3.5 ^c	1.2 ± 0.1 ^c	6.2 ± 0.6 ^a	71.4 ± 9.0 ^b

NANO F3+40% AGAR	4.0 ± 0.2^c	53.3 ± 3.6^b	1.6 ± 0.1^c	5.2 ± 0.2^a	98.0 ± 6.7^a
NANO F3+80% AGAR	2.9 ± 0.4^d	55.7 ± 1.8^b	2.8 ± 0.3^{ab}	4.7 ± 0.4^a	85.4 ± 10.0^{ab}
<hr/>					
AGAR [43]	3.3 ± 0.3	68.3 ± 10.2	7.9 ± 1.2	8.0 ± 0.5	78 ± 10
<hr/>					
PLA [42, 48, 49]	5.1 ± 0.1	44.7 ± 3.8	9.0 ± 1.1	1.3 ± 0.1	77
TPCS [3]	0.5 ± 0.2	6.1 ± 0.7	8.5 ± 3.1	25.2 ± 0.5	47

693

694 As deduced from Table 4, the water vapor permeability (WVP) of the films was also
695 greatly affected by the purification degree of the cellulosic fractions and nanocrystals, as
696 well as by the addition of agar. The less purified fraction (F2A) displayed the lowest
697 barrier performance, which was drastically improved (10-fold reduction in the water
698 permeability) for the nanocrystals produced after the short acid hydrolysis (NANO F2A).
699 This is again related to the removal of amorphous hemicelluloses by the sulphuric acid
700 treatment. Accordingly, the purified cellulose fraction (F3) also showed greater barrier
701 than F2A. In this case, the hydrolysis of F3 had a positive impact on the barrier properties.
702 This could be a combined effect of the slightly greater crystalline character of the
703 nanocrystals as well as the more compacted microstructure of the films (cf. Figure 5).
704 Even though the formation of smaller nanocrystals was detrimental for the mechanical
705 properties of the films, their presence enabled the formation of less porous films, hence
706 inhibiting water diffusion. The WVP of the films from the cellulosic fraction subjected to
707 the longer hydrolysis was also evaluated. As expected, given the reduced crystallinity of
708 the extracted nanocrystals, they produced films with higher WVP $((8.6 \pm 0.4) \cdot 10^{-14}$

Kg·m/s·m²·Pa) than NANO F3, again demonstrating that extending the hydrolysis time did not produce further digestion of the amorphous cellulose domains.

Since agar presented higher WVP than the pure nanocellulosic films, its incorporation was expected to reduce the water barrier performance of the hybrid materials. This was indeed the case for the NANO F2A hybrid films, with the blend containing 80% agar showing a very similar WVP value to that of pure agar [43]. In contrast, the presence of agar in the NANO F3 blends did not have a significant effect on the permeability. This could be explained by a synergistic effect between both biopolymers, i.e. even though the incorporation of an amorphous component such as agar would be detrimental for the barrier performance, the establishment of interactions between agar and cellulose via strong hydrogen bonds would lead to the formation of a stronger and more hydrophobic network, counteracting the negative effect of agar. With regards to similar agar composites previously reported in the literature where nanocellulosic reinforcement ranged from 1% to 10%, WVP results were between 1-2 orders of magnitude higher ($\sim 1.2 \cdot 10^{-12}$ Kg·m/s·m²·Pa) in comparison with those reported in Table 4, probably due to higher nanocellulosic content (20%-80%) and thus showing the optimum barrier performance of TM vine shoots residues when incorporated into agar [46, 47]. Regarding other conventional biopolymers, these composites presented an intermediate barrier between PLA and TPCS [25, 42]. On the other hand, similar materials obtained from *Posidonia oceanica* showed lower barrier when compared with the more purified F3 fraction, but better barrier properties at nanocrystal purification level (both NANO F2A and NANO F3) [6].

To better understand the effect of the incorporation of agar on the water affinity of the hybrid films, contact angle measurements were also performed and the estimated contact angle values are listed in Table 4. While F3 and F2A presented a highly hydrophilic behaviour, which impeded a correct measurement of their water contact angle values, the acid hydrolysis substantially increased the hydrophobic character of the films. As expected, the removal of amorphous components after the acid hydrolysis yielded materials with more hydrophobic surfaces, especially in the more purified NANO F3 film. In keeping with our previous results, a longer hydrolysis treatment led to a slight decrease in the contact angle ($64.7 \pm 7.0^\circ$) of the films, which is reasonable due to the reduced crystallinity of the extracted nanocrystals. On the other hand, the addition of agar produced more hydrophobic surfaces in the hybrid films, which was not unexpected due to the more hydrophobic behavior of pure agar films [43]. Surprisingly, the hybrid films presented similar or even higher contact angle values than pure agar, again suggesting a synergistic effect between cellulose and agar. Other biopolymers such as PLA and TPCS showed similar or even lower values (77° and 47° respectively) to those obtained from TM films and composites demonstrating the high hydrophobicity of these materials and their applicability for food packaging [25, 49].

Although no polyphenols could not be quantified by means of the Folin-Ciocalteu method in the cellulosic fractions and nanocrystals, vine shoots have been previously reported as a phenolic-rich source [12]. Our hypothesis was that the phenolic compounds were actually bound to other components such as hemicelluloses and, therefore, they could not be detected by this colorimetric approach. To assess the bioactivity of the (nano)cellulosic films, a more practical method such as the β -carotene bleaching assay was used and the results are shown in Figure 6. As observed, both of the cellulosic

fractions showed remarkable bleaching inhibition, especially the less purified fraction (F2A), which showed outstanding values of ~50% inhibition after 120 min. However, the antioxidant potential of both fractions was significantly reduced after being subjected to the acid hydrolysis. While NANO F3 did not show a significant antioxidant capacity, NANO F2A still showed a bleaching inhibition of ~30%. These results are much higher than those previously reported for the films from the cellulosic fractions extracted from *Arundo donax* waste biomass (ca. 6% inhibition [50]), thus highlighting the potential of the less purified (nano)cellulosic fractions extracted from vine shoots to develop bioactive materials for food packaging applications.

Figure 6. β -carotene bleaching inhibition kinetics for the films produced from the cellulosic fractions (A) and nanocrystals (B) extracted from *Tempranillo* (TM) vine shoots.

4. Conclusions

In this work, vine shoots have been proposed as alternative sources for the development of sustainable biopolymeric materials for food packaging applications. The composition of two different varieties (*Tempranillo* (TM) and *Verdejo* (VJ)) was firstly characterized. Given their great similarity, TM was selected as the raw material due to its greater worldwide abundance. TM vine shoots were then used to extract cellulosic fractions and

nanocrystals with different degrees of purity. The simplified extraction protocol led to less purified materials (F2A and NANO F2A) with much higher yields and lower crystallinities than those produced by the standard cellulose purification method (F3 and NANO F3). The hydrolysis of F2A produced the digestion of most amorphous hemicelluloses. On the contrary, most of the cellulose microfibril regions in F3 were not accessible to sulphuric acid and the hydrolysis process did not occur uniformly. As a result, NANO F3 was composed of heterogeneous fibrillar structures with different sizes and similar crystallinity to that of F3. Finally, films were made from the cellulosic fractions and nanocrystals in order to evaluate their performance. The digestion of most of the amorphous hemicelluloses in F2A produced a dramatic improvement in the mechanical and water barrier performance of NANO F2A. On the other hand, the more purified F3 fraction produced films with superb mechanical and barrier performance, which were not remarkably improved in NANO F3 after the acid hydrolysis. Interestingly, F2A and NANO F2A films showed a remarkable antioxidant potential, as determined by the β -carotene bleaching assay. Agar was also incorporated at different concentrations (20%, 40% and 80%) into the nanocellulosic films, producing more ductile materials, with greater transparency and smoother surfaces, where the cellulose nanofibrils were embedded in a continuous agar layer. Thus, properties of the hybrid films can be adjusted depending on their intended application by selecting the optimum content of (nano)cellulosic fractions and agar.

These results highlight the importance of evaluating alternative purification protocols which yield less purified materials, since they are not only beneficial from an economical and environmental perspective, but they can generate materials with improved

performance and additional functionalities, such as antioxidant capacity, with interest for food packaging applications.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Figure S1. (A) Visual aspect of the pure (nano)cellulosic films obtained from vine shoots and of their blends with agar. Contact transparency of the pure (nano)cellulosic films (B) and of their blends with agar (C).

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