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RESEARCH

Cellulose-based sustainable packaging of leafy vegetables: an experimental study on the shelf life of baby spinach

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Abstract A novel bio-based and compostable cellulose film (NF) was studied for the packaging of fresh baby spinach, with results compared to a petroleum-derived non-biodegradable polypropylene (PP) film, currently used to market the same product. Baby spinach is a leafy vegetable with high metabolic activity. A preliminary analysis of the product respiration rate was conducted to select the cellulose film grade. The chosen NF film ensures the optimal O_2 and CO_2 concentration in the headspace, performing even better than the conventional PP film. In fact, when the leafy vegetable is packed within PP, after 15 days of storage, no equilibrium value of gas concentration was reached, which, upon longer storage, might cause anaerobic conditions and off-odor development. Baby spinach leaves packed with NF film showed a slower decrement in texture properties and total antioxidant capacity during storage with respect to control samples, but also a larger weight loss, mostly due to the high-water permeability of the cellulose. However, water condensation upon storage was noted for both packaging materials. Analysis of mechanical, thermal, and barrier properties of the NF film before, during, and after use probed no deterioration of material properties, confirming the potentiality of this polymer for sustainable packaging of fresh leafy vegetables.

Keywords Bio-based polymers · Food quality · Shelf-life · Fresh vegetables · Baby spinach · Modified atmosphere packaging · Biodegradable polymers · Compostable polymers

Introduction

The global food demand is continuously rising and is expected to increase by 110% in 2050 (Mc Carthy et al. 2018). This would require an increase in food production, as well as a reduction of food waste. The latter is a critical issue, because about 30–50% of the food produced globally for human consumption is lost or wasted (Myers et al. 2017; Porat et al. 2018), with the largest fraction of food losses constituted by fresh produce. The latter makes up about 45% by weight of the overall food waste (Lipinski et al. 2016), mostly due to postharvest losses (Batziakas et al. 2020; Beretta et al. 2013; Porat et al. 2018).

This paper “Cellulose-based sustainable packaging of leafy vegetables: an experimental study on the shelf life of baby spinach” is dedicated to the memory of our friend and colleague Mario Malinconico.

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Fresh fruits and vegetables naturally deteriorate after harvest, and the rate of deterioration is affected by a variety of factors, including respiration rate, ethylene production, compositional changes, water loss, physiological disorders, and pathological breakdown (Kader 2013). Quality loss can result in the disposal of the commodity, or reduce its consumer acceptability (Shewfelt 2002), which is one of the main drivers of postharvest food waste in fresh produce (Baldwin 2002).

A properly designed food packaging system can prevent fresh produce from being spoiled (Batziakas et al. 2020; D'Aquino et al. 2016; Khan et al. 2021; Torrieri et al. 2009a), thus playing an essential role in the global economy. By controlling and modulating gas and vapor exchanges with the external atmosphere, adapted to food needs, plastic packaging contributes to preserving food quality during storage and improving safety by preventing food-borne diseases or food chemical contamination (Angellier-Coussy et al. 2013). Significant benefits also include reduction of food waste thanks to shelf-life extension (Matar et al. 2018; Verghese et al. 2015), as well as energy and fuel savings, and reduced greenhouse gas emissions from freight transport, thanks to the low weight of plastic packaging.

Despite these huge advantages, plastic packaging is often considered an additional economic and environmental cost rather than an added value, mostly due to the absence of a circular plastic economy and the leakage of millions of tons of plastics, which contribute to environmental pollution and trigger immense economic costs. Development of sustainable packaging, made of bio-based and biodegradable materials as an alternative to fuel-sourced plastics (Di Giuseppe et al. 2022; Di Lorenzo 2021; Di Lorenzo and Androsch 2019; Dobrzyńska-Mizera et al. 2022; Khan et al. 2021; Moeini et al. 2021; Pellis et al. 2021) may not only help to limit plastic waste but also may provide opportunities to participate in a market that is predicted to show a double-digit percentage growth rate within the next 5 years (Kodua 2022).

Different types of bio-based and biodegradable films are available, but their suitability as food packaging materials needs to be addressed for each specific type of food. This holds especially true for modified atmosphere packaging (MAP), where actively respiring produces are sealed within polymeric films, able to control O_2 and CO_2 and water vapor levels within the package atmosphere (Vermeulen et al. 2018). MAP has been shown to reduce postharvest losses by extending the shelf life and maintaining the quality of a variety of fruits and vegetables (D'Aquino et al. 2016; Domínguez et al. 2016; Kader et al. 1989; Khan et al. 2021; Mampholo et al. 2015; Torrieri et al. 2009a; Zhang et al. 2006).

In this contribution, we present the possible exploitation of a bio-based and home-compostable cellulose-based film for the packaging of fresh-cut baby spinach. Cellulose is a linear polymer formed from repeating units of cellobiose and is the most common organic polymer, representing about 1.5×10^{12} tons of the total annual biomass production, and an almost inexhaustible source of raw material (Kaplan 1998; Klemm et al. 2005). Biodegradation of cellulose is provided by bacteria and fungi, with enzyme oxidation occurring specifically by peroxidases secreted by fungi (Klemm et al. 2005). Cellulose and its derivatives have been widely studied as a suitable material to be used in food packaging, but only in blends with other polymers, such as poly(lactic acid), poly(vinyl alcohol), alginate, chitosan, starch, among others (Cazón and Vázquez 2021; Khosravi et al. 2020; Liu et al. 2021). Only a few studies have focused on the use of pure cellulose for packaging films (Yaradoddi et al. 2020; Zahan et al. 2020; Zhao et al. 2019), but to our knowledge, no literature data are available on the use of cellulose-based films for packaging of leafy vegetables, like baby spinach (Shaikh et al. 2021).

Minimally processed baby spinach (*Spinacia oleracea L.*) is a very perishable leafy vegetable with a shelf-life of only 7 days when stored at 7 °C (Tudela et al. 2013). Baby spinach leaves are usually stored and marketed in polypropylene bags (Bergquist et al. 2006; Oliveira et al. 2016), which are not biodegradable, nor compostable, and their use implies considerable environmental implications.

Only a few environmentally friendly polymers have been tested to date for MAP of baby spinach leaves. Poly(lactic acid) (PLA) film has been used to pack fresh-cut spinach. Results showed that although PLA was able to preserve the spinach's flavor during storage, the product shelf life was limited by microbial contamination (Botondi et al. 2015). Antimicrobial film based on olive flounder bone gelatin and zinc oxide nanoparticles showed antimicrobial activity against *L. monocytogenes* inoculated on spinach, without affecting the quality of spinach for 7 days at 4

°C (Beak et al. 2017). Moreover, an antibacterial film comprised of sweet potato starch, montmorillonite nanoclay and thyme essential oil reduced the population of *E. coli* and *S. Typhi* on fresh baby spinach leaves to below detectable levels, within 5 days of storage at 4 °C (Issa et al. 2017).

The objective of this work is to test the suitability of a cellulose film, bio-based and home compostable, for packaging baby spinach, with shelf-life data compared to a conventional, non bio-based, nor compostable packaging film made of isotactic polypropylene, already used to market this product. Experimental analyses are presented and discussed in this manuscript, to assess on one side preservation of appearance and the nutritional quality of the packaged baby spinach leaves, and on the other side, estimate the performance of the cellulose film after prolonged storage of the vegetable.

Materials and methods

Materials

Baby spinach (*Spinacia oleracea* L.) of Italian origin, were kindly provided by AMICO BIO (Santa Maria Capua Vetere, CE, Italy). Tray and Polypropylene film (PP) (30 µm) were furnished by Coopbox (Bibbiano, RE). A NatureFlex™ NF30NVS (NF) cellulose lacquered film with a thickness of 30 µm, was kindly provided by Futamura (Futamura Chemical Co, Ltd., Japan). This NF grade is certified to meet both European EN13432 and American ASTM D6400 standards for compostable packaging (Dukalska et al. 2013).

Respiration rate measurement and packaging design

To select the packaging material able to ensure an optimal gas composition inside the package of the baby spinach leaves, the respiration rate of fresh samples was studied at 5 °C in air, by using a closed system (Torrieri et al. 2009a). The samples (0.1 kg) were placed in a steel jar (0.004 m^3) and conditioned overnight at a temperature test (equilibrium time). The temperature and relative humidity inside the jar were monitored by means of a data logger (Escort Data Login Systems Ltd, Naples, Italy). An air pump was attached to a water humidification system (Torrieri et al. 2009c), which was connected to the inlets of the chamber containing the samples during the equilibrium time. After equilibrium, the inlet and outlet valves were closed and the gas composition was monitored over time with an O_2/CO_2 gas analyzer (accuracy of 0.1%), equipped with a needle (Checkmate 9900, PBI Dansensor, Ringstead, Denmark). The experimental time was 48 h at 5°C. The respiration rate was calculated as reported by (Torrieri et al. 2010) and expressed as mol of O_2 or CO_2 consumed or produced per unit of time and weight.

The ideal film must have an O_2 transmission rate (OTR) through the film (cc h^{-1}) equal to the rate of O_2 consumed by the product (Vr_{o2}) (cc h^{-1}) to assure an optimal equilibrium O_2 partial pressure inside the headspace of the packaging. The same holds for CO_2. The optimal gas composition for the baby spinach leaves was reported to be characterized by low concentrations of O_2 (5%) and CO_2 (5%) to avoid off-odor development and reduce respiration rate (Tudela et al. 2013). Thus, this atmosphere composition was used to solve the oxygen mass balance around the package for identifying the optimal permeability of the film (Torrieri et al. 2009b):

$$\text{OTR} = \frac{\text{Vr}_{\text{O2}} \bullet W \bullet x}{A \bullet \left(P_{\text{O}_2}^{\text{out}} - P_{\text{O}_2}^{\text{in}} \right)} \quad (1)$$

Where W is the weight of the sample (kg), x is the thickness of the film, A (m^2) is the surface area of the film, $P_{o_2}^{out}$ is the partial pressure (atm) of the oxygen in the atmosphere and $P_{o_2}^{in}$ is the partial pressure (atm) of the O_2 in the package headspace at equilibrium. The packaging surface area available for gas exchange was about 864 cm^2.

Baby spinach process and storage condition

Baby spinach leaves were processed the day after the harvest at AMICO BIO company. The samples were washed with water and dried in a tunnel where hot air (80 °C) was circulated for 15 min. Then, 100 ± 5 g of product was placed inside a polypropylene tray of 1150 cc. Samples were subdivided into two lots. One lot was packed with a flexible NF film and the other lot with a flexible PP film (as control), by using a horizontal form-fill-seal packaging machine (Flow pack Orange 580, GNA, Italia). The sealing temperature of the films was set at 150 °C. Samples were weighed before and after packaging and then quickly transported to the laboratory in 30 min. Samples were stored in a refrigerator at 5 ± 1 °C for up to 15 days. The changes in physical and chemical properties of spinach were analyzed after 1, 5, 9, 12, and 15 days of storage. All treatment samples were measured in triplicates.

Chemical analysis

Gas analysis: O_2 and CO_2 concentration (% v v^{-1}) in the package headspace was monitored by means of a portable PBI Dansensor A/S (Check Mate 9900 O_2/CO_2; Ringsted, Denmark) analyzer (accuracy ± 0.1%). Samples of 3 mL of headspace gas were taken through a septum (a patch of silicone sealant applied to the film) using a syringe with a 25-gauge needle.

pH determination: pH was measured on samples obtained by mixing homogenized baby spinach leaves with water (ratio 2:1) by using a pHmeter (Eutech Instruments Pte Ltd. Ayer Rajah Crescent Singapore). The pHmeter was equipped with a Schott electrode which was previously calibrated with buffer solutions (pH 4 and pH 7) at 20 °C. Four measurements were carried out on each sample.

Total phenolic content (TPC): Folin-Ciocalteu reagent was used to determine TPC. The lyophilized sample (0.5 g) was crushed with mortar and pestle with 10 mL of 6% sodium bicarbonate. The homogenate was filtered through a paper filter and 0.5 mL of the filtrate solution was added with 2.5 mL of Folin-Ciocalteau reagent and 2 mL of sodium bicarbonate. The samples were incubated for 1 h at 35 °C and then for 1 h at 6 °C. After 2 h of incubation in the dark, the absorbance was read at 760 nm against a blank (2.5 mL of Folin-Ciocalteau reagent and 2.5 mL of sodium bicarbonate), using a spectrophotometer UV-VIS (UV-550 Jasco, Japan). The total phenolic content was calculated based on the calibration curves of gallic acid (0–8 mg mL^{-1}) and expressed as mg of gallic acid equivalents for grams of dry matter (mg GAE $g^{-1}{}_{dm}$).

Total antioxidant capacity (TAC): TAC was studied by evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Lyophilized samples (0.5 g) were added in 10 mL of methanol/water (80:20). After, the sample with the solvent was put on a platform oscillating for 60 min, then it was placed on an ultrasound bath for 30 min. The sample was centrifuged (Hermle Z 326 K, Germany) at 10,000 rpm for 15 min. The pellet was discarded, and the supernatant was retained and used as the extract (100 µL) that was mixed with 4.9 mL of DPPH solution (methanol + DPPH 0.1 Mm) to initiate the reaction. The absorbance was read using a spectrophotometer UV-VIS (UV-550 Jasco, Japan) at 515 nm after 30 min of incubation at room temperature in the dark. Antioxidant activity was calculated as mg of Trolox equivalents for grams of dry matter (mg TE $g^{-1}{}_{dm}$) using a Trolox standard curve (0–625 mg mL^{-1}).

Physical analysis

The amount of water transmitted over the packaging film was gravimetrically measured by weighing the whole package at the start and at the end of the storage period. The weight loss of the spinach leaves was gravimetrically determined by weighing the product at the initial storage time and after each storage time and was expressed as moisture loss with respect to the initial weight (%). The water absorbed by the packaging film was gravimetrically determined by measuring the increment of film weight at different storage times. The amount of water condensed on the film was measured by weighing the film before and after wiping off the water accumulated on it (Volpe et al. 2018).

The color of the baby spinach leaves was measured with a tristimulus colorimeter (Minolta CR-300, Ramsey, NJ, USA) with a circular measurement area (D = 8 mm). The colorimeter was calibrated using a white standard plate (L = 100). The L^*, a^*, and b^* values were measured on the product surface. Five readings were carried out on each leaf and 15 leaves were measured for each package (Garrido et al. 2015).

The texture was measured by a compression-shear test with a Kramer shear cell consisting of a five-blade probe on a universal testing machine (model TA.TX.plus texture analyzer, Stable Micro Systems, Godalming, UK) equipped with a 100 N load cell. A total of 15 g of leaves were placed in the Kramer shear cell perpendicularly oriented to the blades. The test was performed at a 4.5 cm distance using a test speed of 2 mm s^{-1}. Force–deformation relationships were analyzed, and results were expressed as maximum force (More et al. 2022).

Infrared spectroscopy

All the films were analyzed before and after use with spinach, with samples taken at different times. A PerkinElmer FTIR Spectrum 100 (PerkinElmer Corporation, Waltham, MA, USA) equipped with a PerkinElmer ATR accessory with a diamond crystal was used to analyze surfaces on the outer and inner surface of the films. Spectra were recorded as an average of 16 scans and a resolution of 4 cm^{-1} (Bonadies et al. 2019; Longo et al. 2022).

Thermal analysis

To evaluate the thermal properties of the films, thermogravimetric analysis (TGA) was carried out using a Pyris Diamond TG-DTA analyzer (Perkin Elmer, Waltham, MA, USA). Each sample was heated from room temperature to 100 °C at 10 K min^{-1}, maintained at this temperature for 20 min, and then heated at 10 K min^{-1} to 700 °C. Analyses were conducted under air atmosphere (Vyazovkin et al. 2014).

Mechanical properties

Tensile tests were performed using an Instron Model 5564 dynamometer (Instron© Illinois Tool Works Inc. Norwood, MA, USA) equipped with a 1 kN load cell in tensile mode and a clamp separation rate of 5 mm min^{-1} (Di Lorenzo et al. 2007; Di Lorenzo et al. 2019). Prior to testing, the dumbbell-shaped samples were conditioned at 25 °C and 50 % relative humidity for 48 h (Agustin-Salazar et al. 2020).

Wettability

The wettability of the surfaces (both internal and external) was evaluated by measuring the contact angle (θ). Static contact angle measurements were obtained by the drop shape analysis using a MicroDrop® (First Ten Angstroms Inc., Italy) contact angle meter with a high-speed framing camera (Agustin-Salazar et al. 2020). Uniform volume drops of deionized water (3 µL) were placed on a horizontal film surface with a syringe at room temperature. The contact angle was evaluated using FTA1000 ManualDrop Shape Analysis Software 2.0 version (FTA Inc. Portsmouth, VA, USA) from the drop shape, by measuring the angle formed between the substrate surface and the tangent drawn from the edge of the drop (Tummala et al. 2012; Basile et al. 2015). The contact angles were measured on both sides of the films, with values reported as an average of 3 measurements.

Barrier properties

The water vapor transmission rate (WVTR) was determined using the "cup method" according to ISO 7783 (ASTM 2014). The samples were preconditioned in a chamber set at 25 °C with a relative humidity of 50% until reaching the equilibrium conditions (7 days) (Agustin-Salazar et al. 2020). The experimental environment consists of a cylindrical vessel filled with water and sealed with the studied film. The weight variation of the system was monitored over time.

Statistical analysis

The effect of package film on the weight loss, water absorbed by the film, water condensed in the package, water transmitted through the package, and quality indices were studied by ANOVA using SPSS v 17.0 for Windows (SPSS, Milan, Italy). Data from film characterization were analyzed by one-way analysis of variance using OriginPro 8.5 software (©OriginLab Corporation, Northampton, MA, USA.). Significant differences among the means were tested using Tukey's test. Experimental data were the means ± standard deviation (SD) of three parallel analyses. Significant differences were defined at $p < 0.05$.

Results and discussions

Respiration rate measurement and packaging design

The gas composition inside the package of a fresh respiring product, like spinach, depends on the dynamic interaction between the packaged product and the film used for the packaging. Due to the respiration process, the product consumes the oxygen available in the headspace of the package and produces carbon dioxide. Due to this process, a gas partial pressure difference at the film interface is generated, activating the flux of gas through the film packaging. Thus, the gas composition inside the package depends on the respiration rate of the packed product and the gas permeability of the film. The respiration rate of the baby leaves at 5 °C is equal to 15 ± 1 cc kg^{-1} h^{-1}. By solving the mass balance reported in Eq. (1), the theoretical value of the O_2 transmission rate of the film must be 4.05×10^{-8} cc $m^{-1}Pa^{-1}$ h^{-1}, which corresponds to a value of 1.62×10^{-3} cc m^{-2} Pa^{-1} h^{-1} for a film of 40 µm. The theoretical value was compared to the permeability value reported on the technical sheet of different NF films. The value reported in the technical sheet was measured at a relative humidity of 50%, whereas in real storage conditions, the relative humidity inside the package is close to 100%. Hence, it was hypothesized as an error due to the different conditions. Based on this assumption, an NF with a permeability to oxygen of 5 cc m^{-2} 24 h^{-1} (23 °C and 50% RH; 40 µm) was chosen as film with the permeability to O_2 close to the theoretical value.

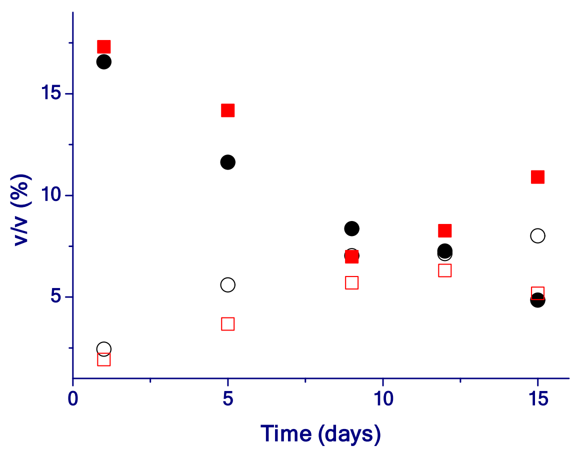

Fig. 1 Head space composition (O_2%, CO_2%) of baby spinach packed with NF (O_2■, CO_2□) or PP (O_2 •, CO_2 ○) film, during storage at 5 °C

Changes in the headspace gas composition during storage of packed baby spinach

The headspace gas composition of packed baby spinach is shown in Fig. 1. For samples packed with control film (PP), the O_2 decreased during storage up to a concentration of 4% after 15 days, whereas the CO_2 increased up to a concentration of 8%. Moreover, after 15 days of storage, no equilibrium value was reached, hence it was possible to predict a continuing change of the gas composition for longer storage time, which can cause an establishment of anaerobic conditions and off-odor development. This demonstrates that PP film is not the ideal material for the specific product, despite it is currently used for its marketing. For samples packed with NF, the O_2 and CO_2 concentrations reached an equilibrium value after 9 days of storage, respectively, of 8 and 6%. The O_2 equilibrium value was slightly higher than the theoretical one (5%), whereas the equilibrium CO_2 value was equal to the optimal one for the baby spinach. Indeed, carbon dioxide around 5% is required to avoid off-odor development (Tudela et al. 2013). Thus, the results of headspace gas composition confirmed that the chosen NF film has the appropriate gas permeability for packaging the baby spinach.

Changes in chemical-quality attributes during storage

The pH of the baby spinach samples is reported in Table 1. Statistical analysis showed that storage time and packaging film have no significant effect on pH ($p > 0.05$) and that it takes an average value of 6.5 ± 0.2 ranging from a minimum of 6.0 to a maximum of 7.0.

TPC does not vary significantly over time for both control and NF samples (Table 1). Statistical

Table 1 pH, TPC, and TAC of baby spinach leaves stored within PP and NF films

Time (days)	PP pH	NF pH	PP TPC (mg GAE g_{ss}^{-1})	NF TPC (mg GAE g_{ss}^{-1})	PP TAC (mg TE g_{ss}^{-1})	NF TAC (mg TE g_{ss}^{-1})
0	6.4 ± 0.0	6.4 ± 0.0	9.0 ± 1.0	9.0 ± 1.0	1.7 ± 0.5	1.7 ± 0.6
1	6.6 ± 0.1	6.6 ± 0.1	9.7 ± 0.3	8.0 ± 1.0	1.5 ± 0.1	1.1 ± 0.2
5	6.5 ± 0.3	6.6 ± 0.1	8.0 ± 2.0	8.2 ± 0.6	1.4 ± 0.1	1.1 ± 0.2
9	6.5 ± 0.0	6.6 ± 0.2	9.9 ± 0.9	8.2 ± 0.4	1.3 ± 0.2	1.1 ± 0.3
12	6.5 ± 0.1	6.5 ± 0.1	6.0 ± 1.0	9.5 ± 0.9	1.2 ± 0.2	0.7 ± 0.1
15	6.5 ± 0.0	6.7 ± 0.1	8.0 ± 1.0	9.0 ± 3.0	1.2 ± 0.3	1.0 ± 0.1

analysis showed no significant differences ($p > 0.05$) between the samples, which assume an average value of 9 ± 2 mg GAE g_{dm}^{-1}.

TAC decreases from an initial value of 1.70 ± 0.04 mg TE g_{dm}^{-1} to a value of 1.01 ± 0.08 mg TE eq g_{dm}^{-1} after 15 days for control samples and 1.2 ± 0.3 mg TE g_{dm}^{-1} for NF samples (Table 1). Statistical analysis highlighted a significant effect of storage time ($p < 0.05$) and packaging film ($p < 0.05$) on the total antioxidant capacity of the baby spinach. In details, baby spinach packed with NF film showed a slow decrement of TAC during storage with respect to control samples. More et al. (2022) correlated the behavior with the decrement of vitamin C.

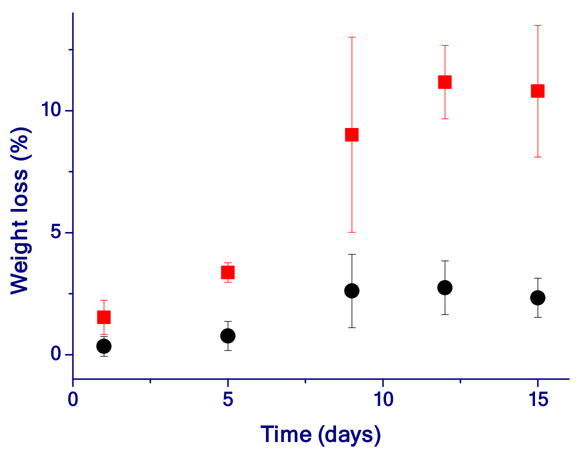

Fig. 2 Weight loss of samples packed with PP (●) and NF (■) film and stored for 15 days at 5 °C

Changes of physical quality attributes during storage

During storage, the amount of water lost from the product due to transpiration (weight loss) is divided into three aliquots: condensed water inside the package, transmitted water through the film, and water absorbed by the film. Figure 2 shows the weight loss of samples during storage at 5 °C. The weight loss of control samples increased slowly up to a value of 2% after 9 days of storage, whereas for samples packed with NF, it increased up to 11% after 11 days of storage.

Figure 3 represents the distribution of the water loss by the product into the three aliquots of condensed water, transmitted water, and absorbed water. The water condensed into the package was higher for samples packed with PP (2 g) with respect to samples packed with NF (0.5 g). Although the NF samples accumulated less water, it was condensed mainly on the film packaged so it was clearly visible (Fig. 4). Instead, for control samples, all the water accumulated in the bottom part of the tray, thus, was less visible. For NF, the water not condensed was partially absorbed by the NF film (2 g) and partially transmitted (8.6 g). The differences are related to the different water vapor permeability of the two films.

The images in Fig. 4 shows also that there were no differences between the two samples in terms of the color of the leaves. The absence of differences was also confirmed by the instrumental color parameters (Table 2) that did not show any statistically significant changes during storage and among samples ($p > 0.05$).

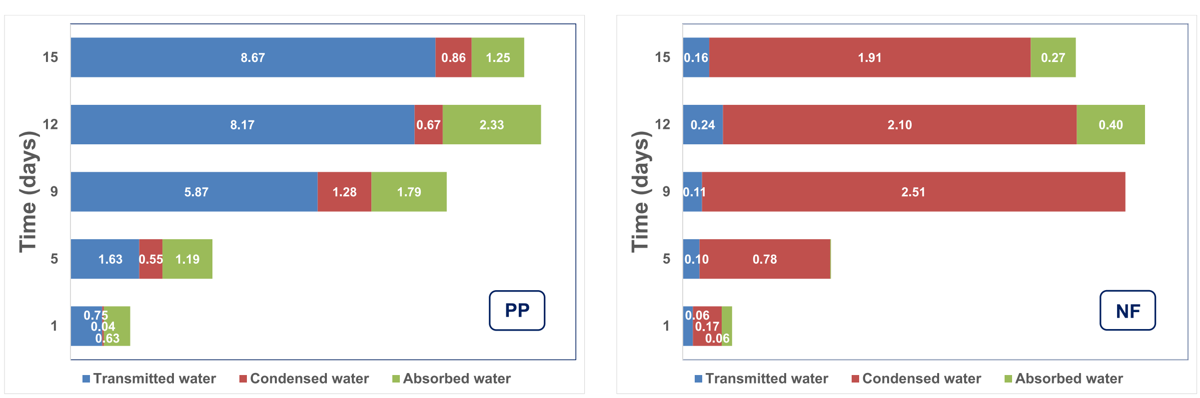

Fig. 3 Transmitted, condensed, and absorbed water in PP and NF packages at different storage time at 5 °C

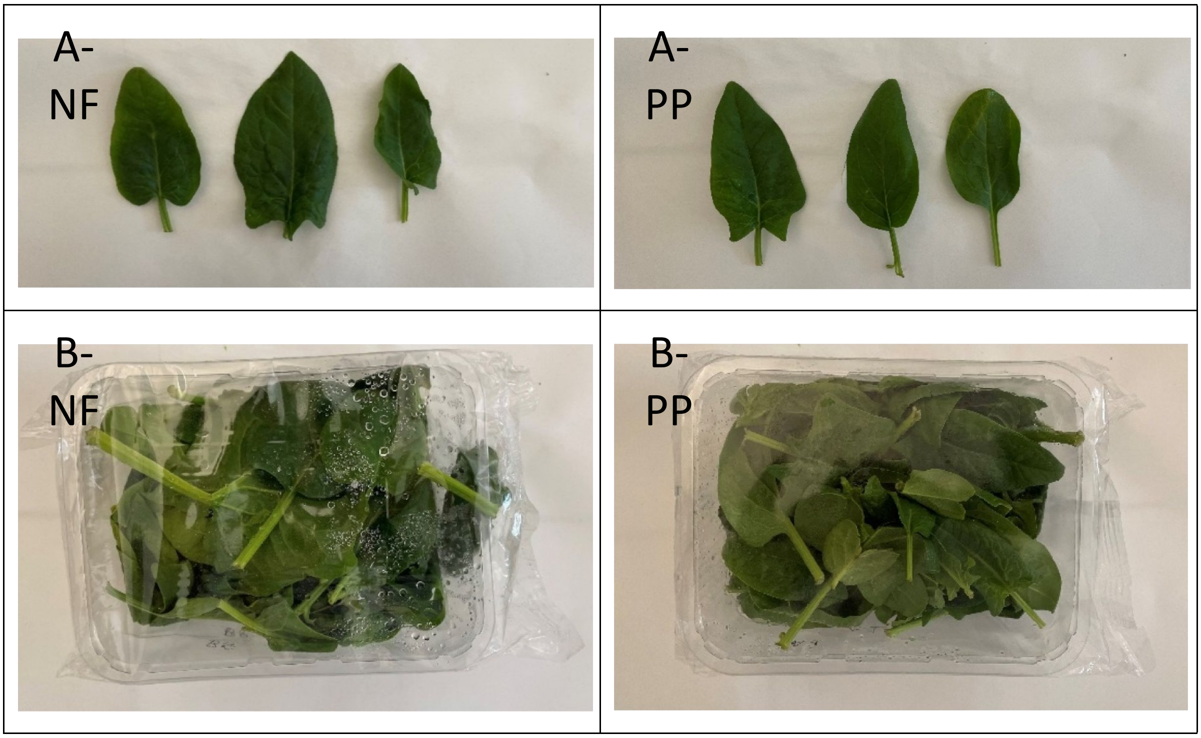

Fig. 4 Baby spinach leaves (**A**) and package samples (**B**) images of the samples stored for 15 days at 5 °C

Table 2 Color parameters (L^*, a^*, b^*) and texture (Force, N) of baby spinach leaves stored within PP and NF films

Time (days)	PP L^*	NF L^*	PP a^*	NF a^*	PP b^*	NF b^*	PP N	NF N
0	50.4 ± 0.2	50.4 ± 0.3	−7.3 ± 0.4	−7.3 ± 0.5	6.4 ± 0.6	6.4 ± 0.7	20.0 ± 4.0	20.0 ± 4.0
1	50.3 ± 0.5	50.8 ± 0.5	−6.7 ± 0.3	−7.7 ± 0.3	5.6 ± 0.2	7.1 ± 0.4	15.0 ± 2.0	14.0 ± 3.0
5	48.0 ± 2.0	49.8 ± 0.6	−7.0 ± 0.3	−7.3 ± 0.8	7.0 ± 2.0	7.0 ± 1.0	16.0 ± 1.0	15.0 ± 2.0
9	50.0 ± 3.0	48.5 ± 0.5	−9.0 ± 1.0	−8.1 ± 0.5	7.0 ± 2.0	6.6 ± 0.8	15.0 ± 3.0	18.0 ± 3.0
12	47.7 ± 0.3	48.5 ± 0.6	−8.3 ± 0.7	−8.8 ± 0.9	7.0 ± 1.0	8.0 ± 1.0	17.0 ± 5.0	18.0 ± 7.0
15	49.0 ± 0.1	48.6 ± 0.8	−7.9 ± 0.3	−8.6 ± 0.3	7.6 ± 0.4	7.8 ± 10.6	17.0 ± 5.0	16.0 ± 5.0

Color changes are usually related to the degradation of chlorophyll over time, causing the leaves to lose their bright green color and turn darker green or yellow. Chlorophyll degradation is caused by an enzymatic reaction (chlorophyllase) that converts chlorophyll into pheophytin (Conte et al. 2008). Moreover, differences in color could also be related to leaf water content as more hydrated samples showed higher color vividness (Garrido et al. 2015). However, although the spinach showed different weight loss due to water transpiration, thanks to the high relative humidity in the headspace, their water content was almost constant during this stage (89%), which rationalizes the above results.

Table 2 also reports the results of the texture of samples over time. Statistical analysis showed that both film and time have a significant effect on the texture properties of the samples ($p < 0.01$). From the data, it is evident that the maximum force decreases from a value of 20 ± 4 N at time 0 to an average value of 17 ± 5 N at time 15 for the PP samples and to 16 ± 5 N for the NF samples. Several factors can be responsible for the texture changes in baby spinach leaves during storage, including microbial activity. Our data agree with the results presented by Medina et al. (2012) and Babic et al. (1996) who reported a decrease in texture of spinach leaves after 2 days of storage at 10 °C. In general, the continuous metabolic activities and moisture loss during storage may be the cause of texture degradation. However, samples packed with NF showed less texture degradation with respect to control samples.

Film characterization

The cellulose film was analyzed before and after use, in order to evaluate possible deterioration of material properties due to use. This allows us to assess the suitability of the bio-based compostable film for packaging fresh baby spinach leaves, and at the same time, find out possible weak points of the material that may allow us to envisage possible strategies to improve material performance, e.g., avoid water condensation on the film surface, as seen in Fig. 4. Comparison of the film quality and features, before and after contact with the fresh leafy vegetable, was conducted only for the NF film, being the PP film already used by Amico Bio company to package and market baby spinach, hence its performance has already been assessed and optimized.

The wettability of the NF films was estimated by measurement of the contact angle, with data displayed in Fig. 5. Measurements were performed on the surfaces in contact with baby spinach leaves (internal), as well as on the external surface of the package. The water contact angle (CA) of the non-used film (0 days) is close to 90° on both sides, which indicates that the film has quite hydrophobic surfaces (Egodage et al. 2017; Ščetar et al. 2017), despite cellulose being a hydrophilic material (Bedane et al. 2015). The hydrophobicity of the film is provided by thin lacquer layers deposited on both sides to improve barrier properties and heat-sealing. In fact, it was possible to observe that for a cellulose film of comparable thickness, but without surface coating, made by the same producer (Natureflex^{TM} NF23NP), contact angle measurements are even not possible, because the film immediately absorbs water.

For the films used for packaging baby spinach leaves, a small, but sizable decrease of the contact angle value can be seen in Fig. 5, with CA

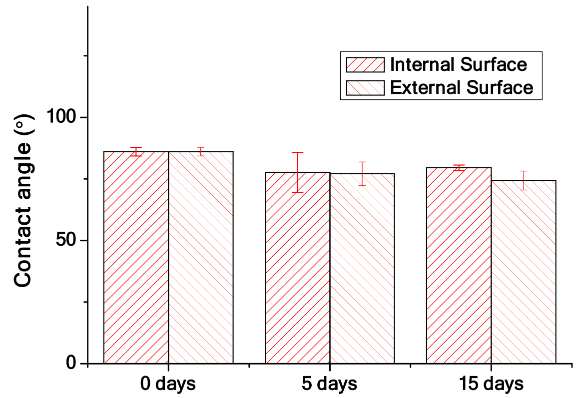

Fig. 5 Contact angle of internal and external surface of NF films

that decreases from 86 to about 77° after use. The improved wettability of the NF film might be caused by moisture absorption, or by partial damage of the coating, possibly due to local deformation of the film occurring during the packaging process, or upon handling. Commercial PP films have CA of about 90–100° (Egodage et al. 2017; Glaser et al. 2019; Ozcalik and Tihminlioglu 2013; Ščetar et al. 2017), higher than the lacquered cellulose film, hence lower wettability. This results in the absence of condensed water on the PP film surface used for packaging baby spinach leaves, as seen in Fig. 4, whereas the lower hydrophobicity of the NF leads to the formation of small water droplets on the internal surface of the NF packaging.

Barrier properties of NF films were quantified as water vapor transmission rate (WVTR) and presented in Fig. 6 as a function of the storage time of baby spinach leaves. WVTR of the virgin film is in agreement with the literature data (Rapisarda et al. 2020; Volpe et al. 2018). Prolonged usage of the film leads to an increase in the WVTR, which does not seem to display a clear trend with storage time. The varied WVTR may be ascribed to partial, local wear of the film coating, which results in higher wettability of the film surface, as probed by the lowered contact angle values, and in turn, increased permeability to water vapor.

The surface properties of the films were analyzed by FTIR-ATR spectroscopy. Figure 7 shows the FTIR-ATR spectra of all films, before and after use with vegetables. All plots display a broad band between 3550 and 3000 cm^{-1}, due to the vibration

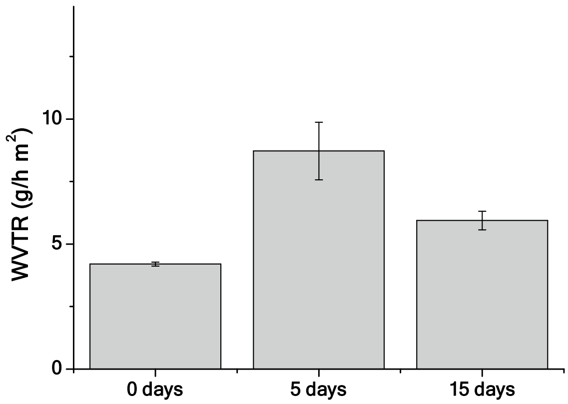

Fig. 6 Water vapor transmission rate of NF film as function of storage time

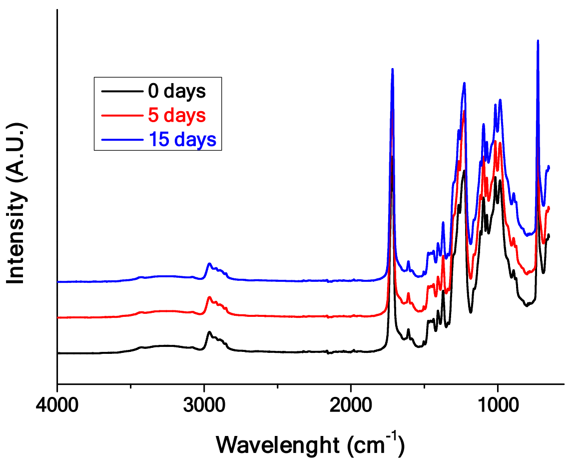

Fig. 7 FTIR-ATR spectra of NF films before and after use as packaging. Curves are shifted vertically for the sake of clarity

of hydrogen-bonded OH-groups; other bands typical of cellulose appear in the 3000–2800-cm^{-1} range, due to C-H and C-H_2 asymmetrical and symmetrical stretching vibration respectively, a peak at 1717 cm^{-1} linked to C=O vibration, and peaks at 1267 and 1228 cm^{-1} due to C-H deformation and C-OH out of plane deformation, respectively. Additional bands appear at 1098, 1075, 1016, and 985 cm^{-1}, due to C=O and C=C ring vibration (Rapisarda et al. 2020; Schwanninger et al. 2004)**.** More importantly, no significant variation of the FTIR-ATR spectra due to the use of the cellulose film for packaging baby spinach leaves appears, as the curves shown in Fig. 7 are practically overlapping.

Thermal stability and water absorption were measured by thermogravimetry, with results compared in Fig. 8 for the film before and after usage. As cellulose films naturally absorb water, NF films were maintained at 100 °C for 20 min to quantify the amount of absorbed water, then heated at a controlled rate (10 K min^{-1}) until decomposition. All plots display three different stages of mass losses, as typical for cellulose films (Torgbo and Sukyai 2020). Below 200 °C mass loss is caused by evaporation of residual water or moisture present in the matrix, which causes rearrangement of the macromolecular structure by disrupting the intermolecular hydrogen bonds (Torgbo and Sukyai 2020). More marked mass loss is observed during the second stage, where degradation reactions occur between 250 and 350 °C, due to the degradation of cellulosic materials, which involves depolymerization of glycoside units and decomposition into monomer of D-glucopyranose (Shim et al. 2019; Torgbo and Sukyai 2019). The final stage above 350 °C is associated with char oxidation, breakdown of carbonaceous residues, and formation of gaseous products with low molecular weight (Vasconcelos et al. 2020), with no residual ash or minerals, as probed by the complete mass loss at high temperatures.

Isothermal at 100 °C for 20 min leads to water release of 7–8 %, for all samples, with minor differences that are within experimental uncertainty. Further heating results in additional water loss, which varies from an overall 12% for NF films not used for packaging to

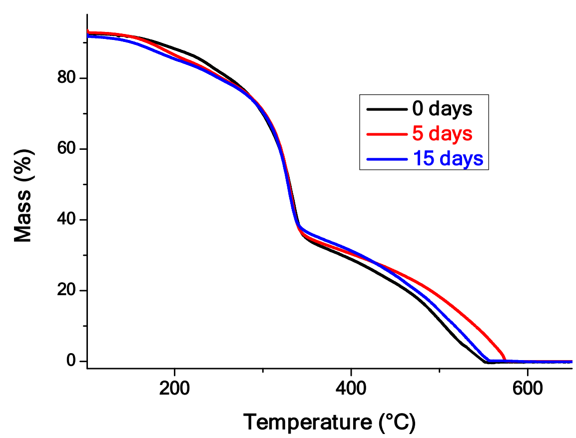

Fig. 8 Thermogravimetric plots of NF films used to pack baby spinach leaves for the indicated times; measurements were performed under air upon heating at 10 K min^{-1}, after 20 min isotherm at 100 °C

14 and 15% for NF films used to store baby spinach leaves for 5 and 15 days, respectively. The higher water uptake of the used cellulose films parallels the varied contact angle of the film surface, as well as the changes in barrier properties of the films. Once the residual water is fully released, around 250 °C, the thermal stability of the films seems not to be affected by storage, as all the plots practically overlap in the temperature range where major degradation due to depolymerization takes place, with minor differences noticed only at very high temperatures. In other words, no significant variation in thermal stability and thermal properties of the cellulose film was observed after use.

Conclusions

Theoretical and experimental analyses were conducted to evaluate the suitability of a bio-based compostable cellulose film for packaging fresh baby spinach leaves, with data compared to a commercial polypropylene packaging film, already used to market this product. The cellulose film showed potential application for the quality preservation of baby spinach, and in some aspects performed even better than the PP film. The latter induces a non-optimal headspace composition of the packed baby spinach, with O_2 and CO_2 concentrations that do not reach equilibrium values even after 15 days of storage. This can result in the establishment of anaerobic conditions and off-odor development upon prolonged storage. Conversely, the chosen NF film was found to have the appropriate gas permeability for packaging the baby spinach leaves, with O_2 and CO_2 concentrations that attain equilibrium values after 9 days of storage, reaching levels suitable to avoid off-odor development. Similarly, samples packed with NF showed minor texture degradation with respect to those packed within the PP film.

The major differences between the two types of films were noted with respect to water loss. The high water vapor permeability of cellulose results in sizable weight loss due to the transpiration of baby spinach leaves, and partial condensation of water droplets on the internal top surface of the package, despite the presence of the lacquer. Using the polypropylene film, water accumulates in the bottom part of the tray, resulting in less visible, but also leading to partial damage of the packaged product, especially the leaves placed at the bottom of the tray.

A comparison of material properties before and after use confirmed the potential application of the chosen cellulose film for the quality preservation of fresh baby spinach leaves. However, to reduce the weight loss and the water condensed on the film, water barrier properties and surface proprieties may need to be optimized, e.g., by properly tailoring the lacquer type or thickness.

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Author contribution S.A.S., B.I., and E.T. conducted experimental analyses; E.T. and M.L.D.L. wrote the main manuscript text; S.A.S, E.T., and M.L.D.L. prepared figures. All authors reviewed the manuscript.

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Data availability We have read the Springer journal policies on author responsibilities and submit this manuscript in accordance with those policies. All of the material is owned by the authors and/or no permissions are required.

Declarations

Conflict of interest The authors declare no competing interests.

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