RESEARCH ARTICLE



Antifungal and ultraviolet–visible barrier properties in starch films reinforced with CuO nanoparticles

João Otávio Donizette Malafatti^{1,2} | Marcelo Ávila Domingues^{1,3} | Mariana Rodrigues Meirelles^{1,4} | Laiza Gabriela Sanches Peres^{1,3} | Joana Dias Bresolin² | Elaine Cristina Paris¹

¹National Nanotechnology Laboratory for Agriculture (LNNA), Embrapa Instrumentação, São Carlos, Brazil

²Department of Chemistry, Federal University of São Carlos, São Carlos, Brazil

³Department of Materials Engineering, Federal University of São Carlos, São Carlos, Brazil

⁴Institute of Chemistry, University of São Paulo, São Carlos, Brazil

Correspondence

Elaine Cristina Paris, National Nanotechnology Laboratory for Agriculture (LNNA), Embrapa Instrumentação, 1452 XV de Novembro St., São Carlos, SP 13560-970, Brazil. Email: elaine.paris@embrapa.br

Funding information

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES); São Paulo Research Foundation (FAPESP), Grant/Award Number: 21/14992-1 and 19/09485-3; Embrapa, Grant/Award Number: 11.14.03.001.01.00; Financiadora de Estudos e Projetos (FINEP); Sistema Nacional de Laboratórios em Nanotecnologias (SisNano); Rede de Nanotecnolgia Aplicada ao Agronegócio (AgroNano Network)

Abstract

Starch materials are subject to loss of initial characteristics due to the retrogradation degenerative effect, high hygroscopicity, and favoring the growth of the microorganisms, which reduces the shelf life of packaging. In this sense, the insertion of nanoparticulated CuO was evaluated for the best performance. CuO is a semiconductor that can improve optical, mechanical, and antimicrobial properties, enabling excellent promotion of starch films. The hot injection precipitation method was used to obtain CuO nanoparticles in the nanometric scale rapidly. The films were carried out from the starch homogenization in water and urea under temperature, followed by thermopressing at 120°C. As a result, the 1% CuO (w w⁻¹) increased tensile strength from 0.87 ± 0.40 to 1.92 ± 0.09 MPa. Furthermore, the films containing nanoparticles showed a barrier property against radiation in the ultraviolet-visible spectrum, not observed for the micrometric scale. Such results were attributed to the superior opacity of the films generated by nanometric reinforcement. Concerning antimicrobial activity, the films containing CuO showed a fungistatic effect (33%) for the Alternaria alternata fungus, a microorganism very susceptible to carbohydrate-rich sources. Thus, the CuO-reinforced starch films improved physicochemical and biological properties, making them promissory candidates for commercial application.

K E Y W O R D S

antifungal, copper oxide, cytotoxicity, fertilizer, optical, packaging

1 | INTRODUCTION

Biopolymers obtained through renewable sources have been studied as an alternative to polymers from petroleum refinement due to the lower environmental impact and energy cost. *Starch* is a polysaccharide obtained from natural sources such as potato, corn, and cassava, presenting a sizeable residual amount available from agricultural processing, besides being biodegradable and biocompatible.¹

Starch biopolymer features high sensitivity to water due to the presence of hydroxyl groups along the polymer chain. In addition, starch films have adversities in the face of mechanical responses and sensitivity to water compared to polyolefins. One challenge is controlling the retrogradation phenomenon, which causes a mechanical performance loss and other initials physicochemical characteristics due to the crystallinity increase over time.² Thus, reinforcement materials can better stabilize and respond to retrogradation, increasing mechanical and hydrophilicity properties.^{3,4}

The particle insertion efficiency depends on the interaction of the reinforcing filler matrix with the polymer, concentration, size, and shape of added particles. In this sense, materials on the nanometer scale have a high potential due to their smaller size and larger surface area, characteristics that facilitate energy transfer with the polymer.^{5,6} Among the ceramic materials, highlight mineral clays such as montmorillonite, zeolites, silica, graphene, and oxides (CuO, ZnO, TiO₂).⁷⁻¹² In addition to increasing the intrinsic properties of the polymer and more outstanding durability, these materials can favor the appearance or intensify properties such as ultraviolet-visible radiation barrier, antimicrobial, and electrical or magnetic conductivity.¹³⁻¹⁵ On the other hand, starch-based materials are pretty susceptible to bacteria and fungi growth due to the high energy source available (glucose), which causes a decrease in the viability of packaging materials due to the contamination susceptibility and the shorter shelf life.¹⁶

Copper oxide (CuO) is a compound with a cubic structure. It can be obtained with stable physicochemical properties, high surface area, and distinct morphologies.^{17–20} In addition, it is a lower-cost candidate than silver nanoparticles in antimicrobial activities, which may allow the preservation of starch films and longer shelf life.^{21–23}

The authors found bactericidal activity similar to silversupported films in the unique paper about starch films reinforced with CuO particles.⁷ However, the literature found no studies regarding the CuO antifungal activity in starch films. Fungi are one of the main microorganisms that degrade carbohydrates-base materials due to their metabolic activity. In this way, the starch films proposed in the present work become an alternative and an advance in the investigations, aiming to gain mechanical resistance and maximum performance, increasing the commercial viability and the biopolymer shelf life due to less degradation by microorganisms.

2 | MATERIALS AND METHODS

2.1 | Materials

The CuO nanoparticles and starch films were obtained from the following precursors: copper acetate (Acros Organics, 98%) and corn starch (Amidex 3001, Ingredion). Synth produced sodium hydroxide, stearic acid,

Applied Polymer_WILEY 2 of 14

acetic acid, and urea. Malt-agar extract (Acumedia) and Tween 80% (Sigma-Aldrich) were used in biological analysis. Commercial CuO (CuO-micro) was supplied by Heringer Fertilizers (Brazil).

2.2 | CuO nanoparticles

The CuO nanoparticles (CuO-nano) were synthesized by the hot precipitation method based on Zhu et al.²⁴ This method enabled the formation of centers for rapid nucleation and particle growth control. In a volumetric flask containing 300 mL of deionized water, 0.038 mol of copper acetate was added and homogenized at room temperature with magnetic stirring. Then, 1 mL of acetic acid was added to stabilize and control the oxidation of Cu^{2+} ions with the temperature increasing. The solution was subjected to the heating process in a glycerin bath at 80°C. The NaOH pellets were quickly added to the solution to obtain a 1 mol L^{-1} concentration. Subsequently, the formation of a black precipitate was instantly observed. The solution was cooled and separated from the reaction medium by centrifugation at 10,000 rpm for 10 min at a temperature of 10°C until a pH close to 8. The precipitate was dried in a circulation oven at 50°C for 24 h.

2.3 | CuO characterizations

2.3.1 | The X-ray diffraction

The X-ray diffraction (XRD) structural analysis verified the particles' phase formation and purity. The diffractograms were obtained from Shimadzu[®] equipment, model LabX XRD-6000 using Cu-K α radiation of $\lambda = 1.5406$ Å, 20 from 5 to 85° at the scanning speed of 1°min⁻¹.

2.3.2 | Field emission scanning electron microscopy

Field emission scanning electron microscopy (FE-SEM) was used to elucidate the particles' size distribution and shape from the images obtained in a JEOL microscope (model 6701F). Image J software verified the mean diameter values of the obtained particles.

2.3.3 | N_2 physisorption/desorption

The textural analysis made it possible to evaluate the pore's surface area, size, and volume with the N_2

^{3 of 14} WILEY_Applied Polymer.

physisorption/desorption isotherms using the Brunauer, Emmett, and Teller (BET) method in MicroMetrics equipment (model ASAP 2020).

2.3.4 | Zeta potential

The second hydration layer charge was found from the zeta potential, making it possible to evaluate the stability of the particles in a colloidal system. The results were obtained using Malvern Instruments equipment Zetasizer (Nano ZS90 model).

2.3.5 | Cytotoxicity

This test was performed to simulate the interaction of the particles with plants in a natural environment, using a methodology adapted from Pignatelli et al.²⁵ The test consisted of the germination of seeds of watercress (Lepidium sativum Linn), selected visually, with color homogeneity and preservation of the seed coat. First, CuO-micro and CuO-nano particles were suspended in distilled water (100 mg L^{-1}). Then the seeds (50 samples) were inserted into a Falcon-type flask (50 mL) to stay in contact with CuO particles at ambient temperature for 1 h. A seed growth as blank negative was performed in CuO particulate absence. After the exposure period, the seeds were separated from the aqueous medium and placed in a Petri dish containing filter paper, allowing the seeds to germinate for 3 to 4 days (under natural lighting). When the roots emerged, the quantity of germinated seeds was counted, qualifying the viability of the seeds.

2.3.6 | CuO solubility

The solubility assay of the CuO particles (CuO-nano and CuO-micro) was carried out at room temperature to evaluate the release of Cu^{2+} ions as a mineral source of fertilizer. For this, 10 mg of the material and 10 mL of deionized water were added to a 10 mL Falcon-type flask. After the solubilization period, the vial was sent for centrifugation at 8000 rpm for 10 min. Then, an aliquot was removed in periods of 0, 5, 10, 15, and 30 min; 1, 2, 4, 6, and 4 h; and 1, 2, and 4, and 8 days. After that, the aliquots were frozen for later analysis. A flame atomic absorption spectrophotometer (FAAS), Perkin Elmer brand, PinAAcle 900 T model was used to quantify Cu^{2+} ions.

Subsequently, a procedure similar to the one described above was carried out, changing the water with neutral pH by an extractive medium of citric acid 2% (w w⁻¹), thus

making it possible to make available the maximum of Cu^{2+} ions from the samples. For this, 5 mg of the material was added to 50 mL of extractive medium. The superior ratio between the material amount and the solution volume compared to the test with water is due to the more significant amount released, minimizing dilutions for reading in the FAAS and avoiding possible reading errors.

2.4 | CuO-reinforced starch films

The preparation of pure starch films was adapted from Moreira et al.,²⁶ using a homogenization procedure in a Haake-type torque rheometer, followed by thermopressing. The film's precursor formulation for processing contained 28 g of corn starch, 7 g of urea, 0.28 g of stearic acid, and 15 g of water to enable homogenization. Previously, the physicochemical characteristics by dispersion charge and surface area responses were evaluated to elucidate the behavior of CuO-nano and CuO-micro particles. Initially, 0.028 g of CuO particulates, corresponding to 1% (w w⁻¹), were dispersed in the water using a tip ultrasound sonication (brand Branson Digital Sonifier) at 40% amplitude for 1 min. Then, the CuO suspension was homogenized manually with the other dry compounds in a plastic bag, obtaining a homogeneous mass. Next, the mixture was submitted to a Haake-type rheometer and a mixer with roller rotors (PolyLab Rheodrive 4, Rheomix OS4). In the processing, the temperature of 110°C and rotation of 160 rpm was used for 4 min. In the end, the thermoplastic material was pressed in a thermopress heated to 120°C, with 10 tons for 5 min, using aluminum molds to obtain the starch films.

2.5 | CuO-reinforced starch film characterizations

Additionally to XDR structural analysis, as described to CuO particles 2.3.1. item, the following techniques were performed:

2.5.1 | Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy technique was used to observe the vibrations of the molecular groups in the films and evaluate possible structural modifications. The analysis was performed in Bruker VERTEX FT-IR equipment with 32 scans in the 4000 to 400 cm⁻¹ wavelength range and resolution of 4 cm⁻¹.

2.5.2 | Mechanical test

The mechanical test (traction) was carried out in decaplicate according to ASTM D882-97 standard at a displacement rate of 0.8 cm s^{-1} in a Stable Micro System TA.XT Plus texturometer. By this analysis, the behavior of the films under a uniaxial tensile force application supplied the deformation and tension responses.

2.5.3 | Colorimetric assay

The color change measurements of the CuO reinforcement films were performed according to the "Hunter scale" based on the parameters L, a, and b in a Chroma Meter CR-410 equipment (Konica Minolta). The L* parameter indicates the luminosity, based on the amount of reflected light, indicating the number of photons in the range of 0 – black and 100 – white. The parameters a* and b* represent the chromaticity index, with a* being the degree between green (–a) and red (+a) and b* between blue (–b) and yellow (+b). From the parameters, the whiteness index (WI) and total color difference (Δ E) were calculated from the white standard with L* = 100, a* = –1, and b* = 2, following Equations 1 and 2.²⁷

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$
(1)

$$\Delta E = \sqrt{\left(L - L^*\right)^2 + \left(a - a^*\right)^2 + \left(b - b^*\right)^2}$$
(2)

The variation values compared to the original film show a greater degree of color change and the influence of the reinforcing materials.

2.5.4 | Optical properties

Diffuse reflectance spectroscopy (DRS) was used to evaluate the capacity to absorb radiation in the ultravioletvisible range of starch films reinforced with different CuO sources. The analysis was performed using the reflectance mode, in the radiation range between 200 and 1200 nm, at room temperature in a Shimadzu UV-vis model 2600 equipment, where reflectance values were transformed in absorbance coefficient.

2.5.5 | Antifungal activity

The tests were conducted to verify the fungicidal activity of the starch films on the starch films against the

Applied Polymer_WILEY 4 of 14

microorganism Alternaria alternata CCT 1250. This selected fungus is the most common in potatoes, corn, and tubers, rich in the starch carbohydrate macromolecule. Initially, the microorganisms were isolated and replicated in a Petri dish with the culture medium with malt agar extract (MEA), grown in a circulating oven at 28°C for 1 to 2 weeks. Then, spore solutions were prepared from the growth of fungi at a concentration of 1×10^6 spores mL⁻¹, using sterile Tween 80 solution (0.1% v v⁻¹). The concentration of spores was determined using a Neubauer chamber, being realized dilutions until the number of spores was standardized by counting them inside this chamber (Kasvi, Paraná, BR) as described by Guarro et al.²⁸ and Pujol et al.²⁹

After adjusting the spore concentration, growth inhibition halo assays were performed against the Alternaria alternata. In a Petri dish with MEA, 100 µL of the spore suspension was added to the center, and a 2×2 cm film sample was added on top of the spores. At the same time, plates with the positive control (only with fungi) and the negative control (only starch film) were prepared and placed in a circulation oven at 28°C. Fungal growth was monitored on the plates daily for 14 days, and the inhibition halos' size was measured with a caliper's aid. At the end of the analysis, the antifungal properties of copper-reinforced starch films were elucidated. In addition, the difference in the size of the growth zones (micellar diameter), it was possible to determine the fungicide (kill fungi) or fungistatic activity (growth inhibition) present in the active copper oxide films. A Two-way ANOVA model performed statistical analysis according to Turkey's test.

2.5.6 | Stability copper-starch

The stability test was performed based on releasing Cu^{2+} ions in an aqueous medium at room temperature in quintuplicates. The films were added to 500 mL of distilled water and periodically removed (3 mL) for 30 days. These aliquots were centrifuged at 8000 rpm for 5 min to quantify Cu^{2+} ions by flame absorption spectroscopy (FAAS).

3 | RESULTS AND DISCUSSION

CuO nanoparticles used as reinforcement in the starch films were obtained by precipitation with the hot injection method. The XRD diffractograms in Figure 1 indicate that the synthesis made it possible to get CuO pure crystalline structural phase (Cuo-nano), similar to that found in the commercial material (CuO-micro).

The diffractogram suggests the CuO single-phase obtaining (JCPDS n° 048–1548) with a monoclinic

5 of 14 WILEY_Applied Polymer_



FIGURE 1 X-ray diffraction of (a) CuO-micro and (b) CuOnano. [Color figure can be viewed at wileyonlinelibrary.com]



FIGURE 2 SEM images of particles: (a) CuO-micro and (b) CuO-nano. [Color figure can be viewed at wileyonlinelibrary.com]

structure. No additional peaks were observed, indicating the absence of a secondary phase or contamination. The differences were noticed in the broadening peaks compared to both diffractograms (Figure 1a, b). This behavior can be attributed to the synthesis performed via a fast method, interfering in the CuO crystallinity and resulting in expected smaller particle sizes.³⁰

An analysis of the images obtained by the scanning electron microscopy (SEM) technique (Figure 2) shows a particle size difference between the commercial and synthesized material. Commercial CuO showed particles with high values superior to the nanometric scale (<100 nm) with a medium of 156 ± 22 . On the other hand, the synthesized CuO was proven to obtain particles on the nanometer scale with a homogeneous diameter distribution (18 ± 4 nm). The result obtained in the present work corroborates that Sagadevan et al.³¹ applied to CuO particles in the range of 10–32 nm (average value of 20.25 nm) performed by the hot injection method.

Evaluating CuO synthesized (CuO-nano) microscopy images verified undefined shape nanoparticles. Zhu et al.³² observed that copper nitrate as a precursor enabled nanorods aspects, varying the synthesis temperature from 2 to 100°C. However, the authors verified a lower growth of the rods at higher temperatures, attributing to the higher energy that promotes the hydrogen bonds breaking and stabilizing the Cu²⁺ ions in the octahedral complex of $Cu(OH)_6^{4-}$. Dehydration occurs mainly in the axial axes during this stage, leading to the formation of the nanorods, preferably at lower temperatures (lower energy). However, increasing nucleation centers promote a significant reaction rate by increasing temperature and more hydrogen bond breakage. Furthermore, the copper acetate as a precursor may have inhibited the preferential growth since it can be readily adsorbed on different crystalline planes of the nuclei, unlike other anions such as Cl^{-} and (SO_4^{2-}) .³³ Thus, in the present work, the copper acetate as a precursor associated with the acetic acid at a temperature of 80°C by rapid injection of mineralizer favored CuO nanoparticles obtaining without preferential growth.

Comparing the textural analysis of commercial and synthesized CuO particles by N_2 physisorption/desorption (Table 1) were respectively determined the surface area values of 12 and 112 m² g⁻¹. The superior surface area value of the synthetic sample is attributed to the difference in the particle sizes concerning the commercial one (CuO-micro) (Figure 2). This synthetic route allowed the formation of particles with high homogeneity, nanometer size, and high specific surface, promoting a contact area increase.²⁴

Zeta potential analysis was performed to verify the stability of CuO dispersions in water from the repulsion/attraction interactions. The results obtained negative values of -37.4 ± 2.2 mV for the CuO-nano and -19.1 ± 0.8 mV (pH around 7.4) for CuO-micro, as shown in Table 1.

The zeta potential higher value for CuO-nano (-37,4 mV) agrees with the particles' decreased diameter medium that promotes incomplete bonds on the surface at the nanometer scale.³⁴ Usually, particle stability increases with zeta potential values, independent to be a positive or negative charge (pH medium). The zeta potential indicates the surface charge on the second layer with the determined solvent. Therefore, zeta potential values suggest a possible condition of the suspended particles repelling each other and presenting good stability (\pm 30 mV).³⁵ In the case of lower values close to zero, the repulsion is reduced, favoring the particles to agglomerate.³⁶ Thus, the synthesized CuO-nano presented high stability in aqueous suspension, showing a zeta potential value of around -37 mV.

Microscopy images in Figure 3 show the fractured surface film characteristics from a cryogenic fracture. In the cross-section images, bubbles or starch granules are absent in the fracture surface for the pure starch film, a smooth surface (Figure 3a). The result can be attributed to the processing carried out by mixing and homogenization enabled the gelatinization and plasticization of the

TABLE 1N2 physisorption/desorption and zeta potentialvalues.

CuO	Specific surface area (m ² g ⁻¹)	Zeta potential (mV)
CuO-micro	12	-19.1 ± 0.8
CuO-nano	112	-37.4 ± 2.2

Applied Polymer_WILEY 6 of 14

starch polymer chains.³⁷ Both nanocomposite films reinforced with CuO-micro (Figure 3b) and CuO-nano (Figure 3d) showed a roughness aspect. This aspect can be attributed to residual starch crystals after processing and CuO containing between the polymeric chains.³⁸ EDS images (Figure 3c, e) exhibit Cu element distribution, showing larger dots attributed to agglomeration sites correlated with a lower dispersion of particles during the mass precursor preparation used for rheometer homogeneity. Particles with minor diameter particles and superior potential zeta value (Table 1) as CuO-nano promoted better stability, minimizing agglomeration in CuO-reinforced starch films. This fact, improved the film processing allows better intercalation with the polymeric starch chains.³⁹

XDR and FTIR analysis carried out the structural effects that may have been caused in the starch films with the CuO reinforcement material. Figure 4a illustrates the diffractograms for the starch films verifying an amorphous halo and peaks with 2 θ located at 13.5, 17.7, 19.5, and 22° characteristic of the semicrystalline starch.⁴⁰ No significant changes were found between the commercial CuO-reinforced starch film (CuO-micro) and the pure one. However, for the synthesized CuO-reinforced starch film (CuO-nano), the presence of the most intense peaks, typical of each reinforcement material, is verified, as shown in Figure 1. This result can be attributed to more considerable interaction with the starch chains attributed to an intrinsic chaotropic effect of the plasticizer urea, which increases intramolecular interaction.⁴¹



FIGURE 3 Images of starch films: pure (a), CuO-micro (b), Cu-EDS CuO-micro (c), CuO-nano (d), and Cu-EDS CuO-nano (e). [Color figure can be viewed at wileyonlinelibrary.com]



FIGURE 4 Structural analysis of CuO-reinforced starch films: (a) XRD and (b) FTIR. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 4b shows a wide vibrational band in the region of 927.8 and 1023 cm^{-1} , attributed to the vibrations of the C–O–C bonds present in the glucose chains 1–4, and of the bonds between hydrogen and oxygen present in the glucose rings (C-O-H), respectively.⁴² Furthermore, a band at 1645 cm^{-1} and another intense at 3389 cm^{-1} were attributed to water and hydroxyl groups (O-H). The presence of urea is verified in the region of 1718 cm^{-1} , referring to the vibration of the ester group. It is possible to confirm that in this region, there is an unfolding of vibration with increasing concentration, in addition to a shift to a lower wave number, indicating changes in the interaction of the matrix with the plasticizer.⁴³ In addition, the vibration 991.4 cm⁻¹ is verified for urea, referring to the interaction with starch, and three vibrational modes located at 1659, 1626, and

1453 cm⁻¹, related to the stretching of the C–O bonds, N-H, and C-N, respectively. The bands in the region between 3449 and 3339 cm⁻¹ belong to urea, associated with hydrogen bonding (N-H); vibrations are increasingly evident with increasing concentration of the plasticizer.^{44,45} Thus, it is possible that during the process of plasticizing starch with urea, due to its chaotropic effect, it enabled the formation of interactions between the plasticizer and the starch. In addition, the two vibrations located at about 2900 cm^{-1} , the vibrational region of the CH bond present in the glucose rings, indicate that the chaotropic plasticizer caused changes in the mobility of the polymeric chains. The FTIR spectra did not show the insertion of CuO particles ($1\% \text{ w w}^{-1}$). CuO presents typical vibrational bands in the 500 to 400 cm^{-1} region, attributed to the Cu-O bond, commonly found for metal-oxygen bonds (M–O).⁴⁶ This result can be attributed to the low concentration in CuO-reinforced starch films, associated with the high intensity of the starch vibrational bands masking the presence of CuO.

Colorimetric analysis was performed to understand the effects of the color changes in the CuO-reinforced starch films, as seen in Figure 5 and Table 2. The visible alterations in films indicate the insertion of the CuO particles, causing both color changes and film opacity. Concerning the pure starch film, a color variation for the white background (ΔE) with a value of 25.8 ± 5.2 was observed, resulting in a high whiteness index of 73.8 \pm 5.2. Figure 5a-c indicates that the synthesized CuOreinforced starch films (CuO-nano) suffered the most color and opacity changes. The color changes greater sensitivity for the nanoparticulate CuO film than the micrometric form may be due to the more effective dispersion and homogeneity of the particles in the starch matrix, allowing a better interaction between the polymeric chains and reducing the light passage.

Diffuse reflectance spectroscopy analysis assessed the optical effects of the composite films. The absorbance spectra of pure starch and CuO-reinforced starch films (Figure 6) indicate that only the film with CuO-nano showed a radiation absorbance capacity, covering the ultraviolet to the visible region (200 to 800 nm). This result may be attributed to the high degree of opacity that the CuO-reinforced starch films (CuO-nano) present, as evidenced by the significant change in the colorimetry parameters (Figure 5). In this way, even with low concentration, this reinforced matrix allowed high activity against UV-vis radiation incidence due to the high degree of barrier to the light passage. This result presents a desirable characteristic, minimizing the degradation of both the polymeric matrix and the components of the films, enabling internal storage of the assets, and increasing shelf life.

Applied Polymer_WILEY 18 of 14



FIGURE 5 Starch-based films images: pure starch (a), with CuO-micro (b) and CuO-nano (c). [Color figure can be viewed at wileyonlinelibrary.com]

Film	L	Α	В	ΔΕ	WI
Pure starch	75 ± 5	0.03 ± 0.07	6.3 ± 0.9	25.8 ± 5.2	73.8 ± 5.2
CuO-micro	59 ± 1	-0.89 ± 0.05	9.7 ± 0.1	41.4 ± 1.3	58.3 ± 1.3
CuO-nano	28.1 ± 0.1	2.3 ± 0.1	4.52 ± 0.02	72.1 ± 0.1	27.8 ± 0.1

TABLE 2L*a*b chromaticityindices values for starch-based films.



FIGURE 6 Diffuse reflectance spectroscopy (DRS) analysis of the pure starch and CuO-reinforced starch films. [Color figure can be viewed at wileyonlinelibrary.com]

The CuO-micro starch film does not present a similar response to radiation absorption compared to CuO-nano one. This result can be attributed to a micrometric size that does not favor an equivalent dispersion and homogeneous interaction with the matrix for better light dispersion. On the other hand, the nanoparticulate ceramic fillers present smaller particle diameters, allowing a greater contact area with the polymeric matrix. Nevertheless, the results obtained in the present work were superior to those verified by Peighambardoust et al.⁷ for silver (Ag), ZnO, and CuO-reinforced starch films by the casting method. In their results, the films containing 1% to 5% of CuO did not show visible radiation absorption capacity due to the transparency, with a narrow absorbance range in the 300-400 nm region. Thus, the material obtained in this work presented a greater



FIGURE 7 Tensile strength versus elongation at break (EB) of CuO-reinforcement starch films. [Color figure can be viewed at wileyonlinelibrary.com]

ultraviolet-visible absorbance capacity, resulting in more extended durability since its degradation by radiation is attenuated.

The mechanical tensile strength test was performed to understand the mechanical response of the CuOreinforced starch films, as shown in Figure 7 and Table 3. The commercial CuO (CuO-micro) 1% (w w⁻¹) films indicate a tensile strength increase concerning pure starch from 0.87 MPa to a maximum of 1.77 ± 0.26 MPa. In Table 3, a tensile strength higher value of 1.92 ± 0.09 MPa (CuO-nano) and a lower standard deviation is observed than the film with CuO-micro. According to Sun et al.,³⁹ this improvement is explained by the

^{9 of 14} WILEY_Applied Polymer_

adequate interface between the filler nanometric with the polymeric matrix, favoring the stress transfer. Peres et al.⁴⁷ obtained films of corn starch reinforced with montmorillonite (MMT). The film reinforced with 1% $(w w^{-1})$ MMT showed the best mechanical strength results, increasing the tensile strength from 2.5 to 3.8 MPa and maintaining the deformation at 60% compared to the pure film. In another work, Sun et al.³⁹ obtained films based on corn starch reinforced with CaCO₃ nanoparticles from 0% to 0.5% (w w⁻¹). The authors verified that adding the nanoparticles increased the tensile strength from 1.40 to 2.24 MPa. The increased tensile strength can also be correlated to starch granules in the CuO-reinforcement starch films, as indicated by microscopy images (Figure 3). In the literature, 48,49 starch films containing starch (nanocrystals or nanoparticles) improved the tensile strength. The reason is an interfacial

 TABLE 3
 Mechanical response of starch films containing CuO particles.

Film	Tensile strength (MPa)	Elongation at break (%)
Pure starch	0.87 ± 0.40	133 ± 7
CuO-micro	1.77 ± 0.26	123 ± 16
CuO-nano	1.92 ± 0.09	105 ± 19

bonding that stresses transfer, similar to that between CuO particles in the starch film. Thus, the CuOreinforcement starch results found in this work are according to the expected tensile strength gain and values.

Subsequently, the antimicrobial activity of the CuOreinforced starch films was carried out to evaluate the microorganisms' proliferation resistance that promotes polymer matrix degradation. The antifungal assay was realized using a disk diffusion analysis for the fungus *Alternaria alternata*, commonly found in carbohydraterich foods, such as corn and potato. The results are observed in Figures 8 and 9.

An increasing micellar growth (white color) is observed by the fungus growth over 14 days, indicating the microorganism development with time. In addition, the conidia germination or sporulation stage (black color) is also verified in the reproduction stage. For the positive control (pure fungus), the growth conditions (nutritional culture medium and temperature) promote an effective growth microorganism. A similar result was observed for the negative control, indicating that the pure starch film under the fungus did not inhibit its growth by smothering (lower oxygen availability), demonstrating the *Alternaria alternata* development susceptibility in this condition.

The efficiency of the CuO-reinforced starch films (micro and nanometric scale) allowed a similar delay in





FIGURE 9 Starch films fungistatic behavior over 14 days for *Alternaria alternata* fungus (a) and statistical analysis, using the Two-way ANOVA model according to Turkey's test (b). [Color figure can be viewed at wileyonlinelibrary.com]

the fungus development, verified by the micellar diameter smaller size concerning the negative (pure film) and positive (only fungus) controls. Thus, the CuO in both films allowed the fungistatic activity appearance, which was non-existent for the pure starch film. Furthermore, the CuO made possible a toxicity level that caused partial death and lower development of microorganisms. The values of the growth halos over time can be verified in Figure 9a.

In Figure 9a, the negative and positive controls reached the maximum possible diameter of the Petri dish on the 11th day. The films with CuO presented more accentuated growth curves when compared to controls until the 8th day, demonstrating the lower development degree of the *Alternaria alternata* fungus. The CuO-reinforced starch films showed values within the same deviation range from the fungus sensitivity results and the micellar growth curve behavior.

Applied Polymer_WILEY 10 of 14

In the case of CuO-nano compared to the CuO-micro one, the faster activity can be associated with the greater penetration degree and reactivity of the particles in the fungal cells due to the smaller sizes. After the 8th day, the fungus showed a constant plateau for the CuOreinforced starch films, with similar micellar diameter values around 60 cm. This stabilization indicates that the films with CuO inhibit the fungus growth and proliferation. Comparing the maximum diameter value reached on day 11th (plate size) is verified that the CuOreinforced starch films showed a decrease of about 33%. The antifungal activity for Alternaria alternata proved for CuO films corroborates with the activity found for this microorganism in assays evaluating other free particulates of copper, Cu(OH)₂, and CuSO₄.⁵⁰ Statistical results (Figure 9b) demonstrated no distinction in activity between CuO-reinforced films. Additionally, both films showed statistical significance in controlling the micellar growth diameter of the fungus compared to the pure starch film from the 8th day. Thus, CuO presence had a positive effect on controlling fungal growth, indicating antimicrobial activity.

The antimicrobial activity arising from CuO is attributed to semi-permeability (osmotic balance) and enzymatic protein denaturation in the cell membrane.⁵¹ Internally in fungal cells, copper can promote the production of highly reactive oxidant radicals (Reactive Oxidative Species – ROS), causing structural changes in lipids, proteins, DNA, and nucleic acids that can decrease micellar growth rate, fungi sporulation, and even death.⁵² The antimicrobial activity efficiency is directly linked to the concentration and the antimicrobial material applied, influencing the adhesion and accumulation of particles on the surface of the fungi and, consequently, in the metabolic activities.⁵³

In the literature, ^{54,55} the essential oils are commonly antifungal agents in a biopolymer starch film matrix. Sapper et al. ⁵⁵ obtained a blend via starch casting with gellan gum, an anionic polysaccharide isolated from the *Sphingomonas elodea* bacterium with high water solubility. They evaluated the essential oil extracted from thyme as an antifungal agent for *Alternaria alternata*. In the results, the authors observed that incorporating 0.5 g of oil per g^{-1} of total solids (polymers) was necessary to promote a similar performance to the present work. Thus, the current CuO-reinforced starch films proved to be an alternative with lower energy expenditure as an active agent in low concentration and a more accessible performance.

The level of viability of these seeds was evaluated to quantify the harmfulness of CuO by a germinated amount after exposure to particulates, as shown in Figure 10 and Table 4. The results indicate that the contact during 1 h with 100 mg L^{-1} , did not cause significant

11 of 14 WILEY_Applied Polymer_

MALAFATTI ET AL.



TABLE 4 Development of watercress roots germinated after exposure to CuO particles.

Sample	Seed viability (%)
Negative control (water)	96 ± 3
CuO-micro	93 ± 3
CuO-nano	91 ± 5

seed death since the level of viability of root development was in a range of 90% (Table 4). The result was similar to the control medium using pure water.

In Figure 10, it is possible to verify that the roots after 4 days of germination already had root roots, which are more evident in the control seeds, followed by CuOmicro and CuO-nano. This appearance of root roots indicates proper root development and active metabolic activity. For the CuO-nano particles, this lower shortening of the root length, and similar diameters throughout the root part, demonstrates the lowest degree of development and, consequently, a level of toxicity. A similar result was found in the literature,^{56,57} in which it suppressed root elongation despite not affecting germination. This result may be due to the accumulation of particles on the surface, causing oxidative stress in the plant. One of the main action mechanisms is forming reactive oxidant species (ROS), which can damage DNA, proteins, cell ionic balance, oxidative degradation of lipids and membranes, and even cell death.⁵⁸ The toxicity found for copper sources is due to the solubilization of Cu^{2+} ions and the adhesion of oxide particles on the plant's surface. Perreault et al.⁵⁹ found that both CuO nanoparticles and the Cu²⁺ ion promoted changes in the metabolic activities of lentil plants (48 h of exposure), attributing photosynthesis's deactivation, more excellent thermal energy dissipation, and decreased electron transport. Stampoulis, Sinha, and White⁶⁰ verified that applying CuO nanoparticles at a concentration of 1000 mg L^{-1} reduced about 90% of the biomass in zucchini seeds after exposure and incubation after 14 days. Furthermore, they found that the maximum concentration for plant growth was 0.02 mg L^{-1} . The material cytotoxicity depends on several factors: type, concentration, stability (solubilization), the plant, time exposure, and its growth phase.

One of the advantages of using CuO, in addition to its antimicrobial properties, is that it can be used as a micronutrient for plants, being a source of Cu^{2+} ions mainly at the nanometric scale due to the gain in solubilization capacity. Sha and Belozerova⁶¹ verified an increase in lettuce root growth by 40% and 90% when Cu concentrations of 130 and 600 mg Cu per kg⁻¹ of the plant were applied. Margenot et al.⁶² evaluated the efficiency of using CuO nanoparticles as a source of Cu²⁺ ions for roots. The results showed that nanoparticles of 16 nm in diameter increased growth by 52% and 26% in lettuce and carrot roots, respectively. In this way, CuO nanoparticles become attractive in optimizing the properties of the starch film and contributing to the development of plants when applied in adequate concentration.

From solubilization assays in an aqueous medium at neutral pH and 2% (w w⁻¹) citric acid extractive medium, the capacity of Cu²⁺ ions was evaluated as plant mineral nutrients. Through the solubility results performed in water (neutral pH, 10 mg of fertilizer in 10 mL of aqueous solution) shown in Figure 11 is observed that CuOnano showed a higher release of Cu²⁺ ions with a value of 10 mg L⁻¹ after 8 days when compared to CuO-micro with the respective released value of 1 mg L⁻¹. This result is due to the greater reactivity of the particles in



FIGURE 11 Assay of CuO solubility and release of Cu^{2+} ions: (a) water and (b) 2% citric acid extractive medium (w w⁻¹). [Color figure can be viewed at wileyonlinelibrary.com]

Applied Polymer_WILEY 12 of 14

the nanometer size, associated with the high surface area and dispersion stability, allowing a minor agglomeration, favoring the solubility of the oxide. Likewise, it is noteworthy that the solubility test was carried out in a neutral aqueous medium, releasing about 1% (800 mg L⁻¹) of the maximum expected amount for CuO, even in nanoparticulate form.

The maximum extraction capacity of Cu^{2+} ions from the CuO particulates was verified by a solubility assay performed in an extractive medium of 2% citric acid $(w w^{-1})$ with pH < 2, using 5 mg for 50 mL of solution. Figure 11b shows a higher release of Cu^{2+} ions for CuOnano and CuO-micro, attributed to oxides tending solubility in an acidic medium. For CuO-nano, the maximum amount of release is observed after 1 h. In addition, values above 40 mg L^{-1} (about 60%) were found in the first 5 minutes. Compared to CuO-micro, similar values were only observed after 6 h of release, demonstrating a slower behavior when compared to the nanometric form. Thus, both CuO could release more than 70% of Cu^{2+} ions during 8 days. The higher solubilization rate observed for CuO-nano is attractive once plants absorb the micronutrient in ionic form. However, a more extended period to solubilize the fertilizer increases the probability of losses caused by leaching and complexation in non-absorbable states. Thus, under environmental conditions that allow the solubilization of CuO, it can become a source of mineral fertilization when degraded in the environment.

The stability of the CuO-reinforced starch film was performed by image monitoring for 30 days and the release quantity of Cu^{2+} ions (Figure 12). In addition, the assay was performed for CuO-nano, attributed to the nanoscale properties. Visually, after 30 days, the films



FIGURE 12 The behavior of CuO-reinforced starch films in a neutral aqueous medium for 30 days with the release of Cu^{2+} ions from 0.65 g of material (2 × 2 cm). [Color figure can be viewed at wileyonlinelibrary.com]

13 of 14 WILEY_Applied Polymer

maintained the apparent structure, not significantly destroying the polymeric matrix, even with swelling. Furthermore, at the end of the period, the films containing CuO-nano showed a similar coloration observed at the initial time, indicating high stability in aqueous media attributed to the plasticization of the processed starch.

In this period, Cu²⁺ ion values were constant and inferior to 0.2 mg L^{-1} for CuO-reinforced starch films with 1% CuO-nano (w w^{-1}) (Figure 12). The result exhibits the CuO-reinforced starch films have high stability for maintaining the matrix structure with interaction with CuO particles, not releasing significant material to the external environment before contact that allows a biological degradation and/or extractive medium.

CONCLUSION 4

The precipitation with the hot injection method made obtaining CuO nanoparticles (diameter less than 20 nm) with a high surface area and reactivity possible. The CuO-reinforced films 1% (w w⁻¹) allowed the effective gain in tensile strength. The CuO nanoparticulate was superior to the micrometric form, allowing a superior opacity and absorption capacity at ultraviolet-visible radiation. In addition, the CuO-reinforced films showed antimicrobial activity (Alternaria alternata fungus). The results showed a significant increase in the characteristics of the starch film, making it a more attractive and favorable source of CuO as a fertilizer carrier system.

AUTHOR CONTRIBUTIONS

João Otávio Donizette Malafatti: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); writing - original draft (lead); writing - review and editing (lead). Marcelo Ávila Domingues: Data curation (supporting); investigation (supporting); methodology (supporting). Mariana Rodrigues Meirelles: Data curation (supporting); investigation (supporting); methodology (supporting). Laiza Gabriela Sanches Peres: Investigation (supporting); methodology (supporting). Joana Dias Bresolin: Data curation (equal); methodology (equal); writing - review and editing (equal). Elaine Cristina Paris: Conceptualization (lead); funding acquisition (lead); project administration (lead); writing - original draft (supporting); writing - review and editing (lead).

ACKNOWLEDGMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. São Paulo Research Foundation (FAPESP) (Grant Number 19/09485-3 and

21/14992-1). The authors acknowledge Embrapa (Grant Number 11.14.03.001.01.00), Financiadora de Estudos e Projetos (FINEP), Sistema Nacional de Laboratórios em Nanotecnologias (SisNano), and Rede de Nanotecnolgia Aplicada ao Agronegócio (AgroNano Network) for financial support.

CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ORCID

João Otávio Donizette Malafatti D https://orcid.org/0000-0002-7551-0510

Elaine Cristina Paris b https://orcid.org/0000-0001-8599-9674

REFERENCES

- [1] E. Corradini, C. Lotti, E. S. de Medeiros, A. J. F. Carvalho, A. a. S. Curvelo, L. H. C. Mattoso, Polymers 2005, 15, 268.
- [2] A. Jiménez, M. J. Fabra, P. Talens, A. Chiralt, Food Bioproc. Tech. 2012, 5, 2058.
- [3] J. D. L. Souza, C. G. Chiaregato, R. Faez, J. Polym. Environ. 2017, 26, 670.
- [4] B. Chatterjee, N. Kulshrestha, P. N. Gupta, Phys. Scr. 2015, 90, 25805.
- [5] S. Fu, Z. Sun, P. Huang, Y. Li, N. Hu, Nano Mater. Sci. 2019, 1, 2.
- [6] A. Arora, G. W. Padua, J. Food Sci. 2010, 75, 75.
- [7] S. J. Peighambardoust, S. H. Peighambardoust, N. Mohammadzadeh Pournasir, P. Pakdel, Food Packag. Shelf Life 2019, 22, 100420.
- [8] N. Follain, R. Crétois, L. Lebrun, S. Marais, Phys. Chem. Chem. Phys. 2016, 18, 20345.
- [9] P. C. Belibi, T. J. Daou, J. M. B. Ndjaka, L. Michelin, J. Brendlé, B. Nsomd, B. Durand, J. Food Eng. 2013, 115, 339.
- [10] C. Horst, C. H. Pagno, S. H. Flores, T. M. H. Costa, J. sol-Gel Sci. Technol. 2020, 95, 52.
- [11] E. Arezoo, E. Mohammadreza, M. Maryam, M. N. Abdorreza, Int. J. Biol. Macromol. 2020, 157, 743.
- [12] E. C. Paris, J. O. D. Malafatti, A. J. Moreira, L. C. Santos, C. R. Sciena, A. Zenatti, M. T. Escote, V. R. Mastelaro, M. R. Joya, Environ. Sci. Pollut. Res. 2022, 29, 41505.
- [13] B. Sharma, P. Malik, P. Jain, Mater. Today Commun. 2018, 16, 353.
- [14] V. K. Thakur, M. R. Kessler, Polymers 2015, 69, 369.
- [15] E. C. Paris, J. W. M. Espinosa, S. de Lazaro, R. C. Lima, M. R. Joya, P. S. Pizani, E. R. Leite, A. G. Souza, J. A. Varela, E. Longo, Chem. Phys. 2007, 335, 7.
- [16] S. d. C. Brito, J. O. D. Malafatti, F. E. Arab, J. D. Bresolin, E. C. Paris, C. W. O. de Souza, M. D. Ferreira, Inorg. Nano-Met. Chem. 2022, 53, 490.
- [17] M. R. Quirino, G. L. Lucena, J. A. Medeiros, I. M. G. d. Santos, M. J. C. d. Oliveira, J. Mater. Res. 2018, 21, 21.

- [18] A. Karimiyan, H. Najafzadeh, M. Ghorbanpour, S. H. Hekmati-Moghaddam, J. Zahedan, *Res. J. Med. Sci.* 2015, 17, 2.
- [19] A. M. Raba-Páez, J. O. João, C. A. Parra-Vargas, E. C. Paris, M. Rincón-Joya, *Mater. Today Commun.* 2020, 26, 26.
- [20] A. P. Moura, L. S. Cavalcante, J. C. Sczancoski, D. G. Stroppa, E. C. Paris, A. J. Ramirez, J. A. Varela, E. Longo, *Adv. Powder Technol.* 2010, *21*, 197.
- [21] K. Mageshwari, R. Sathyamoorthy, J. Mater. Sci. Technol. 2013, 29, 909.
- [22] A. Nemati, S. Shadpour, H. Khalafbeygi, S. Ashraf, M. Barkhi, M. R. Soudi, *Mater. Manuf. Processes* 2015, *30*, 63.
- [23] P. Kanhed, S. Birla, S. Gaikwad, A. Gade, A. B. Seabra, O. Rubilar, N. Duran, M. Rai, *Mater. Lett.* **2014**, *115*, 13.
- [24] J. Zhu, D. Li, H. Chen, X. Yang, L. Lu, X. Wang, *Mater. Lett.* 2004, 58, 3324.
- [25] S. Pignattelli, A. Broccoli, M. Renzi, Sci. Total Environ. 2020, 727, 138609.
- [26] F. K. V. Moreira, J. M. Marconcini, L. H. C. Mattoso, Polym. Bull. 2012, 69, 561.
- [27] A. Babaei-Ghazvini, I. Shahabi-Ghahfarrokhi, V. Goudarzi, Food Packag. Shelf Life. 2018, 16, 103.
- [28] J. Guarro, L. Soler, M. G. Rinaldi, Eur. J. Clin. Microbiol. Infect. Dis. 1995, 14, 613.
- [29] I. Pujol, J. Guarro, C. Llop, L. Soler, J. Fernández-Ballart, Antimicrob. Agents Chemother. 1996, 40, 2106.
- [30] R. Wu, Z. Ma, Z. Gu, Y. Yang, J. Alloys Compd. 2010, 504, 45.
- [31] S. Sagadevan, K. Pal, Z. Z. Chowdhury, J. Mater. Sci. Mater. Electron. 2017, 28, 12591.
- [32] J. Zhu, H. Bi, Y. Wang, X. Wang, X. Yang, L. Lu, Mater. Chem. Phys. 2008, 109, 34.
- [33] M. A. Abbasi, Y. Khan, S. Hussain, O. Nur, M. Willander, *Vac-uum* 2012, 68, 1998.
- [34] A. El-Trass, H. Elshamy, I. El-Mehasseb, M. El-Kemary, *Appl. Surf. Sci.* 2012, 258, 2997.
- [35] C. N. Lunardi, A. J. Gomes, F. S. Rocha, J. De Tommaso, G. S. Patience, *Can. J. Chem. Eng.* **2021**, 99, 627.
- [36] J. D. Clogston, A. K. Patri, Methods Mol. Biol. 2011, 697, 63.
- [37] X. Luo, J. Li, X. Lin, Carbohydr. Polym. 2012, 90, 1595.
- [38] L. Dai, H. Yu, J. Zhang, F. Cheng, Int. J. Biol. Macromol. 2021, 181, 868.
- [39] Q. Sun, T. Xi, Y. Li, L. Xiong, PLoS One 2014, 9, 1.
- [40] M. I. J. Ibrahim, S. M. Sapuan, E. S. Zainudin, M. Y. M. Zuhri, *Int. J. Food Prop.* 2019, 22, 925.
- [41] J. F. Mendes, R. T. Paschoalin, V. B. Carmona, A. R. Sena Neto, A. C. P. Marques, J. M. Marconcini, L. H. C. Mattoso,

Applied Polymer_WILEY 14 of 14

E. S. Medeiros, J. E. Oliveira, *Carbohydr. Polym.* **2016**, 137, 452.

- [42] H. Schmitt, A. Guidez, K. Prashantha, J. Soulestin, M. F. Lacrampe, P. Krawczak, *Carbohydr. Polym.* 2015, 115, 364.
- [43] X. Ma, J. Yu, Carbohydr. Polym. 2004, 57, 197.
- [44] N. R. Savadekar, V. S. Karande, N. Vigneshwaran, P. G. Kadam, S. T. Mhaske, *Appl. Nanosci.* 2015, 5, 281.
- [45] S. F. da Costa, J. C. De Andrade, M. M. Meier, S. H. Pezzin, *Materials* 2018, 23, 23.
- [46] C. Yang, X. Su, J. Wang, X. Cao, S. Wang, L. Zhang, Sens. Actuators B Chem. 2013, 185, 159.
- [47] L. G. S. Peres, J. O. D. Malafatti, B. Bernardi, L. H. C. Mattoso, E. C. Paris, *J. Polym. Environ.* **2023**, *31*, 2388.
- [48] L. Dai, C. Qiu, L. Xiong, Q. Sun, Food Chem. 2015, 174, 82.
- [49] X. Li, C. Qiu, N. Ji, C. Sun, L. Xiong, Q. Sun, Carbohydr. Polym. 2015, 121, 155.
- [50] A. A. Malandrakis, N. Kavroulakis, C. V. Chrysikopoulos, Sci. Total Environ. 2019, 670, 292.
- [51] A. La Torre, V. Iovino, F. Caradonia, *Phytopathol. Mediterr.* 2015, 54, 201.
- [52] A. Shoaib, S. Akhtar, N. Akhtar, Glob. Nest J. 2015, 17, 664.
- [53] A. H. Wani, M. A. Shah, J. Appl. Pharm. Sci. 2012, 2, 40.
- [54] D. Kowalczyk, M. Kordowska-Wiater, J. Nowak, B. Baraniak, Int. J. Biol. Macromol. 2015, 77, 350.
- [55] M. Sapper, P. Wilcaso, M. P. Santamarina, J. Roselló, A. Chiralt, Food Control 2018, 92, 505.
- [56] A. Gallo, L. Manfra, R. Boni, A. Rotini, L. Migliore, E. Tosti, *Environ. Int.* **2018**, *118*, 325.
- [57] Z. Wang, X. Xie, J. Zhao, X. Liu, W. Feng, J. C. White, B. Xing, *Environ. Sci. Technol.* 2012, 46, 4434.
- [58] J. Yanga, W. Cao, Y. Rui, J. Plant Interact. 2017, 12, 158.
- [59] F. Perreault, M. Samadani, D. Dewez, Nanotoxicology 2014, 8, 374.
- [60] D. Stampoulis, S. K. Sinha, J. C. White, *Environ. Sci. Technol.* 2009, 43, 9473.
- [61] V. Shah, I. Belozerova, Water. Air. Soil Pollut. 2009, 197, 143.
- [62] A. J. Margenot, D. A. Rippner, M. R. Dumlao, S. Nezami, P. G. Green, S. J. Parikh, A. J. McElrone, *Plant Soil* 2018, 431, 333.

How to cite this article: J. O. D. Malafatti, M. Á. Domingues, M. R. Meirelles, L. G. S. Peres, J. D. Bresolin, E. C. Paris, *J. Appl. Polym. Sci.* **2023**, *140*(33), e54290. <u>https://doi.org/10.1002/app.54290</u>