Photochromic Paper Indicators for Acidic Food Spoilage Detection

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ABSTRACT: A photoresponsive microstructured composite is fabricated through the impregnation of cellulose filter paper (FP) with a spiropyran-modified acrylic polymer. The polymer enwraps uniformly each individual cellulose fiber, increases the thermal stability of cellulose, and ensures the preservation of the composite functionalities even upon removal of the surface layers through mechanical scratching. The photochromic spiropyran moieties of the polymer, even while embedded in the cellulosic sheet, can reversibly interconvert between the colorless spiropyran and the pink merocyanine isomeric states upon irradiation with UV and visible light, respectively. Moreover, the photochromic polymer presents a faster photochromic response and a higher resistance to photodegradation, with an outstanding reusability for more than 100 switching cycles when it is incorporated in the cellulose network. Most importantly, the acidochromism of the modified FP, attributed to the spiropyran molecules after UV activation, allows the real-time optical and visual detection of acidity changes and spoilage in food products, such as wine and milk. Spoilage due to bacterial degradation and oxidation processes generates acidic vapors that induce the protonation of the merocyanine. This results in a visually detectable chromic transition from pink to white of the treated cellulose fibers, corresponding to a blue shift in the absorption spectrum. The developed photoresponsive cellulose composite can serve as cost-effective robust optical component in integrated functional platforms and consumer-friendly indicators for smart food packaging, as well as portable on demand acidoresponsive interfaces for gas monitoring in industrial and environmental applications.

INTRODUCTION

Paper is comprised of a three-dimensional fibrous network of cellulose, the most abundant naturally occurring polysaccharide, extracted from wood pulp through mechanical and chemical processing. Structurally, cellulose is a linear homopolymer consisting of D-glucose units connected by (1 → 4) β linkage, held together by strong inter- and intra-molecular hydrogen bonds between the hydroxyl pendant groups of the glucopyranose rings. Owing to their high specific strength and stiffness, light weight, nontoxicity, abundancy, recyclability, biodegradability, and low cost, paper-based objects, such as containers, bags, strips, filters, and cleaning cloths, continue to attract a growing interest as sustainable alternatives to petroleum derivatives. In the last years, a rapid development of cost-effective paper-based technologies is being registered in the fields of filtering, printing, packaging, and sensing through the incorporation of functional molecules and polymers in the cellulose network. In fact, in this way, additional features and functionalities are introduced to the paper, such as elasticity, adsorptive, or ion-exchange capability, resistance to microbiological attack and thermal degradation, water and oil repellency, magnetic or emitting properties, electrical conductivity, and optical and colorimetric response to pH, metal ions, volatile amines, and proteins.

Recently, the possibility to develop sustainable materials with multiple functionalities that can be activated on demand by means of light stimuli has been explored, by blending polymers and photochromic spiropyran (SP) molecules. In such systems, the photomodulation of the material properties arises from the reversible light-triggered interconversion between two different SP isomers, an apolar colorless SP and a zwitterionic purple merocyanine (MC), which entails physical and chemical variations, such as refractive index, dipole moment, optical absorption and emission, wettability, and chemical recognition. For instance, our group has exploited SP molecules dispersed within polymer films, and electrospun fibers and nanofibers of synthetic and natural polymers to yield mechanically robust platforms with photo-switchable optical, mechanical, and ion or acid gas-sensing...
properties. In other studies, SPs have been embedded in macromolecules from renewable sources, such as polypeptides, chitosan, and cellulose, through covalent linkage. As known, a significant advantage of the covalent inclusion of SPs in a polymer over the simple mixing is the reduction of the SPs aggregation and, thus, the enhanced physicochemical stability and improved fatigue resistance. However, it was reported that the direct functionalization of paper with pending SP moieties by covalent linkage impeded the reversion from the polar MC to the ring-closed SP isomer because of the dimensional constraints imposed by the cellulose cavities and by the highly polar cellulosic environment. Alternatively, to increase the mobility of the photochromic units, the incorporation of polymer networks containing SP moieties through layer-by-layer electrostatic assembly techniques or the covalent attachment on the cellulose fibers substrate have been proposed. However, the fabrication of such systems requires time-consuming multistep functionalization or postprocessing treatments, and the rate of the SP recovery is significantly affected by the strong electrostatic or hydrogen bonding interactions between the MC and the cellulose matrix.

A tremendously important and yet unexplored application of SPs incorporated in paper substrates would be in the smart food packaging area. In fact, the photoactivated acid recognition properties of SPs, combined with the mechanical and chemical stability, nontoxicity, and white contrast of paper, could constitute a cost-effective detection technology that could be directly integrated in the packaging material to allow a practical monitoring of the food state to the end user, through wireless detection or naked eye visualization. In this context, although several types of colorimetric, optical, electrochemical sensors, radio frequency identification tags, electronic noses, and biological and enzymatic assays have been proposed, their use is often impractical for the shelf-life evaluation and the quality control operations in food processing industries because of their complex design, long detection times, high costs, chemical and physical (i.e., temperature, humidity, electromagnetic fields) interferences, time consuming procedures, and uneasy multivariate statistical methods or mathematical modeling for data interpretation.

Moreover, to assess the freshness and quality of fish, meat, alcoholic beverages, and dairy products, much effort has been devoted to the development of chemoresponsive materials, based on fluorescent dyes, modified carbon nanotubes and conductive polymers, and of sophisticated analytical methods (i.e., electrokinetic chromatography, capillary zone electrophoresis) that can target volatile compounds associated with microbial decomposition, such as biogenic amines, rather than acidic byproducts.

Herein, we present a straightforward preparation protocol based on a noncovalent functionalization of paper strips with SP-modified poly(2-hydroxyethylmethacrylate) (SP-PHEMA) upon their immersion in the corresponding polymer solution. In this way, the amount of SP adsorbed onto the paper can be controlled by simply changing the dipping time and the polymer concentration. Moreover, in contrast to the aforementioned works, the interactions between the SP photopolymer and the cellulose matrix do not hinder the conformational freedom of the photochromic moieties, and subsequently, the overall photochromic performance is enhanced with respect to the SP-PHEMA deposited on glass, in terms of photoconversion kinetics and resistance to photodegradation, allowing a high reusability for more than 100 UV–vis switching cycles. Most importantly, in addition to the material’s attractive optical properties, we demonstrate its application as photoswitchable acidochromic sensory probe for food inspection. In particular, through a case study, we demonstrate for the first time that the acidochromic properties of SP polymer adsorbed on paper constitute an efficient and readily available approach to monitor the variations in the acidity of milk and wine, as they enable the real-time optical and visual detection of the acidic vapors that are generated in the deterioration process of these types of food. In this way, it is possible to render paper an ideal acidochromic carrier for the rapid and effective colorimetric detection of spoilage markers in smart packaging applications. In light of its optical and chemical response, the developed composite is suitable for security packaging, smart in-package food indicators for real-time quality control and prevention of health hazards, portable acidic vapor sensing platforms for environmental and industrial monitoring, and several other applications including rewritable paper or anticounterfeit printing.

### RESULTS AND DISCUSSION

The SP-PHEMA copolymer was synthesized using standard radical polymerization procedures. The reaction schemes and chemical characterization are presented in the Supporting Information file (Schemes S1 and S2, Figure S1). The polymer was then adsorbed on filter paper (FP) by impregnation. As shown in Figure 1, the surface morphology of the paper samples before and after the incorporation of SP-PHEMA appeared similar, indicating that the polymer penetrates within the fibrous cellulose network covering each fiber uniformly with a thin layer, consistently with previous observations of cellulose fibers treated with a cyanoacrylate polymer using a similar approach.

To verify the presence of SP-PHEMA onto the FP, chemical analysis of FP/SP-PHEMA was carried out through Fourier transform infrared (FTIR) spectroscopy. As it can be observed in Figure 2, the spectrum of the FP/SP-PHEMA presents the characteristic absorption bands of cellulose, specifically the stretching modes of the OH groups at 3300 cm$^{-1}$, of the C–O at 1030 cm$^{-1}$ and of the carbon ring breathing centered and 1161 cm$^{-1}$, which are comparable to those of the untreated FP, revealing no degradation of the material. Most importantly, the presence of the carbonyl absorption peak of the polymer at 1728 cm$^{-1}$ (inset of Figure 2) indicates that the SP-PHEMA was successfully adsorbed on FP. The other representative bands of the photochromic polymer at 3400, 1160, and 1074 cm$^{-1}$, corresponding to the stretching modes of O–H, C–O and C–O–C, respectively, could not be detected in the FP/SP-PHEMA composite because of the overlap with the strong absorption peaks of the cellulose in those regions.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Optical micrographs of (a) untreated FP and (b) FP impregnated with the photochromic polymer (FP/SP-PHEMA).
The presence of the SP-PHEMA polymer adsorbed on the outermost fibers surface of FP was also confirmed through X-ray photoelectron spectroscopy (XPS). As discussed in the Supporting Information, the binding energy variations of the C 1s peak and its components (Figure S2 and Tables S1 and S2) reveal a possible interaction via hydrogen bonding between the hydroxyl groups of cellulose and the ester groups of the acrylic polymer, similar to what reported for composites of cellulose and polyesters.3

To assess the thermal stability of the developed material and to evaluate the effect of the interactions between the FP and the SP-PHEMA polymer on the thermal decomposition profile of the composite, a thermogravimetric analysis (TGA) was performed. As shown in Figure 3a, the thermal degradation of the untreated FP started above 200 °C and the main mass loss was observed in the 300–400 °C temperature range, as a consequence of the thermal pyrolysis of the cellulose skeleton.35 For the SP-PHEMA, the highest rate of degradation was observed above 400 °C, similar to other acrylate polymers.36 Upon incorporation of 1% wt of SP-PHEMA in FP, the temperatures corresponding to the maximum degradation rates of FP (T1д) and SP-PHEMA (T2д) extracted from the derivative of the TGA curves (Figure 3b) drastically changed from 342 to 353 °C and from 439 to 423 °C, respectively, revealing that the SP polymer impregnation improves the thermal stability of the paper, in agreement with similar effects observed on cellulose-acrylic graft copolymers.35 and, at the same time, that the inter- and intra-molecular interactions between the polymer chains in the SP-PHEMA become weaker. Both effects could be ascribed to the formation of H-bonds between cellulose and SP-PHEMA, which, on the one hand, increases the FP resistance to thermal degradation and, on the other hand, destabilizes the SP-PHEMA polymer–polymer interactions. These findings demonstrate that noncovalent embedding of low contents of acrylic polymers in cellulose significantly improves the resistance of the latter to thermal decomposition, owing to the thermally protective polymer layer formed around each cellulose fiber and, possibly, to stabilizing interactions between the two components. Interestingly, by substantially increasing the SP-PHEMA concentration to 17% wt with respect to the FP, the decomposition profile of the SP-PHEMA component, characterized by the formation of a defined shoulder at 430 °C, approached the value measured for the T2д of the pure polymer, but only a variation of 3 °C (356 °C) with respect to the 1% wt FP/SP-PHEMA was registered in the T1д. This suggests that a concentration of SP-PHEMA of 1% wt is sufficient to coat and thermally protect the cellulose fibers, whereas a higher concentration may form a thicker coating but does not actively contribute to the interactions with cellulose, leading to a minimal contribution to the further increase of the thermal stability of FP.

The incorporation of the SP-PHEMA polymer in FP gives rise to important optical changes upon light irradiation ascribed to the photochromism of the SP units. As observed in Figure 4a, upon UV irradiation the samples turn from white to pink. This change occurred in less than 2 min under the irradiation conditions used in this work. This is due to the photoconversion of the SP molecules to their isomeric form MC, as demonstrated in Figure 4b by the formation of an absorption peak centered at 545 nm in the visible region of the absorption spectrum. The homogeneous color of the sample (Figure 4a) demonstrates that the impregnation process yields uniformly coated surfaces, rendering it a practical and fast technique for the functionalization of paper with photochromic polymers. Moreover, the MC form exhibited a red fluorescence (Figure 4c) with an emission centered at 620 nm, whereas no signal was registered for the SP, consistently with other studies on SP-based composites.16,17 The fluorescence imaging allowed to reveal the SP-PHEMA homogeneous distribution even at the microscale level. In fact, as observed from Figure 4a, the well-defined fibrous morphology of the cellulose network further indicates that the fluorescent SP-PHEMA polymer formed a coating layer around each individual fiber.

Figure 2. FTIR spectra of FP, photochromic polymer (SP-PHEMA) and FP impregnated with the polymer (FP/SP-PHEMA). Inset: Zoom-in of the 1900–1500 cm⁻¹ spectral region.

Figure 3. (a) TGA of untreated FP and FP (FP/SP-PHEMA) containing 1 and 17% wt of SP-PHEMA polymer. (b) First derivative of the thermogravimetric curves of the corresponding samples. The degradation temperatures of FP (T1д) and of SP-PHEMA (T2д) for each sample are indicated in the graph.
Upon irradiation of the previously UV-irradiated sample with visible light, the MC state was reverted to its pristine SP state in 30 s, and the sample recovered its white color. These results demonstrate that the paper substrate does not hinder the conformational flexibility of the SP units attached to the polymer chains, allowing a reversible interconversion between the two isomers.

To further assess the photochromic performance of the developed material and the effect of the interactions with cellulose on the efficiency of the photoconversion of SP, the irradiation times needed for the photoisomerization and the resistance to photodegradation of SP-PHEMA adsorbed on FP were compared with those of a similar amount of polymer deposited on a glass slide. As observed in Figure S3a,b, upon UV irradiation under the conditions used in this work, the maximal photoconversion of SP to MC was reached after 90 s in the paper composite, and after 150 s in the SP-PHEMA cast on glass. In a similar way, the SP form was fully restored after 30 s of visible irradiation in the paper composite and after 40 s in the glass coating. The faster switching rate between SP and MC measured upon incorporation of the polymer in the cellulose substrate may arise from a higher mobility and free volume between the SP-PHEMA polymer chains dispersed in the cellulose substrate, which results in a more efficient photoisomerization. Moreover, the weakening of the inter- and intra-molecular interactions between the SP-PHEMA polymer chains due to the formation of H-bonds between cellulose and SP-PHEMA could further contribute to the photoswitchability of SP because it limits the aggregation of the photochromic moieties.

In addition to the photoinduced recovery process, the spontaneous reconversion of MC to SP in the FP/SP-PHEMA composite and in the SP-PHEMA film cast on glass was investigated by storing the samples in the dark after UV irradiation and the comparison between the two systems is presented in the Supporting Information file (see Figure S4, Table S3 and relative discussion). In addition to the faster photoconversion observed in the case of the paper-impregnated photochromic polymer, a major improvement in its photodegradation was also registered. As shown in Figure 5a,b, the pure SP-PHEMA polymer coated on the glass could not undergo more than 50 photoswitching cycles, and the absorbance peak of MC isomer at 545 nm decreased by \( \sim 20\% \) after the first 20 cycles (Table S4). Conversely, after the impregnation in paper, \( \sim 93\% \) of the initial MC optical signal.

Figure 4. (a) Photographs and corresponding confocal images of FP impregnated with the photochromic polymer (PF/SP-PHEMA) samples before and after irradiation with UV light (the nonirradiated samples appear identical to the ones irradiated first with UV light and subsequently with visible light). (b) Absorption and (c) emission spectra (\( \lambda_{ex} = 560 \text{ nm} \)) of FP/SP-PHEMA before and after UV irradiation. SP and MC refer to the SP and MC forms, respectively.

Figure 5. (a) Absorption spectra of FP impregnated with the photochromic polymer (SP-PHEMA) on glass upon alternate irradiation with UV and visible light for 150 and 40 s, respectively. The subscripts in the legend refer to the corresponding cycle number. SP and MC refer to the spiropyran and merocyanine isomers of the photochromic polymer, respectively. (b) Absorption at 545 nm of SP-PHEMA on glass upon 60 UV−vis cycles. (c) Absorption spectra of FP/SP-PHEMA upon alternate UV and vis irradiation for 90 and 30 s, respectively. (d) Absorption at 545 nm of FP/SP-PHEMA upon 150 UV−vis cycles. 

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was retained after the first 20 cycles (Table S4), and although a progressive fall-off was measured, more than 50% of the intensity was still optically detectable after 110 cycles, whereas the value decreased to 24% after 140 cycles. In parallel, the chromic change in the sample could be observed by naked eye and although the value decreased to 24% after 140 cycles, the intensity was still optically detectable after 110 cycles, whereas a progressive fall-off was measured.

The high resistance to photofatigue can be attributed to the embedding of the photochromic polymer in the paper substrate and to the polar nature of cellulose, which sensibly reduce the formation of MC aggregates and the associated photodegradation effects. These findings demonstrate the robust photochromic response of the composite. To the best of our knowledge, this is one of the very few studies in which the photochromism of SP-based systems is demonstrated for more than 100 cycles. Moreover, such a high reusability in terms of photoswitching cycles has not been previously documented on SP-functionalized paper substrates.

The durability of the SP-PHEMA coating on the FP was assessed by scratching and subsequently imaging the scratched sample areas through confocal microscopy after UV irradiation (Figure S6). As demonstrated in the Supporting Information file, the uniform coating of SP-PHEMA around each cellulose fiber imparts self-similarity. In this way, the material functionalities are preserved even after the removal of surface layers.

To determine whether the combination of the SP-PHEMA with the FP would constitute an effective approach to obtain portable indicators for the optical and visual detection of acidic vapors on demand, the acidochromic properties of the FP/SP-PHEMA composite were investigated by shining UV light either in the same conditions reported for the photochromic cycles or for 5 s, using a portable UV lamp, on two samples used as caps for vials containing liquid HCl and water (Figure 6a). Upon exposure to a neutral environment, such as water vapors, the UV irradiation promoted the SP–MC photoisomerization, giving rise to the expected chromic variation from white to pink in the material (photograph of Figure 6a), as confirmed by the absorption spectrum of MC in Figure 6a. Conversely, the FP/SP-PHEMA sample in contact with HCl did not exhibit a significant chromatic difference after UV irradiation but presented the typical absorption maximum of the protonated MC species (MCH) centered at 415 nm (Figure 6a). This effect is ascribed to the immediate protonation of the formed MC to its MCH counterpart upon exposure to HCl vapors. The blue shift in the MC absorbance maximum from to 545 to 415 nm is a result of the reduction in the electron delocalization that follows after protonation. The obtained color (pale yellow) is very similar to the original white color of the modified FP substrate with SP-PHEMA. Therefore, the rapid acidochromic response coupled to a distinct photoactivated colorimetric detection by naked eye of the MC and MCH species demonstrates the FP/SP-PHEMA sensory functionality. To assess the material potentiality for the detection of acidic vapors associated to milk and wine spoilage, FP/SP-PHEMA smart caps were used to seal vials containing ultrahigh temperature (UHT) and pasteurized milk and three Sangiovese-based red wines. As they deteriorate, these food samples are known to develop acidic vapors (water vapors), whereas no significant change is detected in the sample exposed to HCl because of the protonation of the photogenerated MC to MCH. Inset: Mechanism of the conversion of SP to MC and subsequent protonation to MCH. (b) Photographs of UV-irradiated FP/SP-PHEMA samples as caps of a vial containing UHT milk at day 0 and day 5 and corresponding variation of pH and total acidity of UHT milk samples as a function of time. (c) Photographs of UV-irradiated FP/SP-PHEMA samples as caps of a vial containing table red wine at day 0 and day 1 and corresponding variation of pH and total acidity of table red wine samples as a function of time. The chromic change from pink to white is ascribed to the conversion of MC to MCH, upon exposure to acidic vapors generated during the spoilage process. The increase in the acidity over time is confirmed by the increase in the total acidity values and by the decrease of pH.

Figure 6. (a) Photographs of FP impregnated with the photochromic polymer (FP/SP-PHEMA) samples inserted in hollow screw caps in contact with water and HCl vapors immediately after UV irradiation and absorption spectra of SP, MC, and protonated MC (MCH) in FP/SP-PHEMA. After UV irradiation for 90 s (λ = 320–375 nm, power density = 4.15 mW·cm−2) or for 5 s (λ = 365 nm, power density = 5 mW·cm−2), the pink color corresponding to the conversion of SP to MC can be visually observed in the absence of acidic vapors (water vapors), whereas no significant change is detected in the sample exposed to HCl because of the protonation of the photogenerated MC to MCH. Inset: Mechanism of the conversion of SP to MC and subsequent protonation to MCH. (b) Photographs of UV-irradiated FP/SP-PHEMA samples as caps of a vial containing UHT milk at day 0 and day 5 and corresponding variation of pH and total acidity of UHT milk samples as a function of time. (c) Photographs of UV-irradiated FP/SP-PHEMA samples as caps of a vial containing table red wine at day 0 and day 1 and corresponding variation of pH and total acidity of table red wine samples as a function of time. The chromic change from pink to white is ascribed to the conversion of MC to MCH, upon exposure to acidic vapors generated during the spoilage process. The increase in the acidity over time is confirmed by the increase in the total acidity values and by the decrease of pH. Samples. Specifically, as shown in the insets of Figure S7a,b, in the photographs of Figure 6b,c and in Video S1, the pink color was no longer detectable after 5 days in both milk samples and after only 1 day in all the red wine samples. Furthermore, the spectral analysis confirmed that the samples cannot be colored...
due to the protonation of MC to MCH, similar to what observed upon exposure to HCl vapors (Figure S7a,b). In parallel, the pH and the total acidity of the food samples were measured for 10 days to investigate the relation between their chemical modifications and the abovementioned response of the FP/SP-PHEMA indicator. As shown in Figures 6b and S8a, the pH of the pasteurized and UHT milk samples went from initial values of 6.70 and 6.75 to 6.60 and 6.62, with a decrease of 1.50 and 1.90%, respectively, within 5 days. At the same time, the total acidity, expressed as lactic acid, reached values above 0.16% in both types of milk in 5 days, with a variation of 22% with respect to their initial values (Figures 6b and S8b). As previously reported, the pH and acidity of unspoiled milk are approximately 6.70 and 0.14%, respectively, and are mainly associated with citric acid, phosphates, carbon dioxide, and casein.38 A pH of 6.60 or lower and acidity values above 0.16% on aging are due to the production of lactic acid by bacterial action.36,38

Conversely, in the red wine samples, the most significant changes in the acidity were detected in the first 2 days. Specifically, the main decrease (1.0–2.6%) in the pH was measured after one day, whereas the total acidity, expressed as tartaric acid, increased by 2.5–6.0% after 1 day and reached a plateau at day 2, with an overall variation of 5–10% depending on the wine (Figures 6c and S9a,b). Such variations with respect to the initial state were previously attributed to an early stage of wine modification.39 Interestingly, notable differences in the acidity and pH profiles could be observed among the three wines. In particular, after 2 days, the table wine T exhibited the highest variation with respect to the initial values in both pH (~3%) and acidity (10%) (Figure 6c), whereas changes of 1.5–1.8% for pH and of 5–8% for acidity were detected from the analysis of the wines with a certified designation of origins N and C (Figure S9a,b). The different changes in the pH and total acidity of the wines might depend on their alcohol content because their composition is similar (Sangiovese grape). Specifically, the fastest increase in the acidity and decrease in the pH were measured in the table wine, which has a lower alcohol content compared with the other wines. As alcohol is effective in inhibiting bacterial growth,39 the wines with a higher alcohol concentration possibly undergo spoilage processes more slowly with respect to the table wine, consistently with the obtained results.

Overall, these findings clearly demonstrate the strong correlation between the variation of the analytical parameters relative to the acidity and the color/optical response of the FP/SP-PHEMA composite. In fact, the chromic variation observed at day 1 and at day 5 in FP/SP-PHEMA caps on wine and milk samples, respectively, indicates that the FP/SP-PHEMA system is sensitive to changes of 1.0 and 1.5% in the pH and of 2.5 and 22.0% in the total acidity of wine and dairy products, respectively. Although in milk such changes are clearly indicative of ongoing alteration and deterioration processes, further studies are needed to assess whether the abovementioned modifications in wines registered in the first 2 days are effectively diagnostic of a compromised state of the wine due to bacterial or sensory spoilage. However, the results demonstrate that the FP/SP-PHEMA composite can reveal differences in the acidity with respect to the pristine values measured immediately after opening the wine bottles, thus providing information about the freshness of wine against frauds and counterfeiting. Moreover, as shown in Figure S10, by plotting the absorbance ratio of the MCH (415 nm) and MC (545 nm) species as a function of the total acidity, expressed as lactic and tartaric acid in milk and wine, respectively, a linear dependence was found ($R^2 = 0.990$) for a concentration range of 0.135–0.175% for lactic acid and 5.10–5.65 g/L for tartaric acid, indicating that the optical response of the composite could be used for the quantitative determination of the acidity upon spoilage.

Another important feature of indicators is their selectivity toward the target analytes. Upon testing the composite against vapors of representative volatile spoilage markers of milk (ethanol, acetaldehyde, lactic acid, acetic acid)40,41 and wine (acetaldehyde, acetic acid and ethyl acetate),42 chromic and spectral changes were observed exclusively in the presence of vapors of acetic and lactic acid for all selected exposure times ranging from 60 s to 10 days (Figure S11). These results clearly demonstrate that the system response is selective toward acids.

Regarding the reversibility of the response, after the exposure to vapors of organic acids and food samples the MC form of the indicator could not be restored by thermal desorption or light irradiation. This might be ascribed to a slow desorption of the acid vapors due to strong interactions with both the polar cellulose substrate and the MC units of the photochromic polymer. However, such irreversible response would not hinder the functionality of the developed composite, as it is intended as a single time use disposable indicator that could be easily integrated into food packaging materials. Therefore, the developed material could serve as a low-cost in situ food quality indicator system, enabling the optical and visual detection of changes in acidity and, thus, acidic spoilage. Such systems could represent an alternative to time-consuming sensory analysis based on color, taste, and flavor carried out by panels of trained experts, a commonly used technique to assess the quality of wine and dairy products.37 Moreover, they would be especially beneficial for quality control operations in the food industry and for consumers, as they would constitute an easily accessible tool integrated in the package to rapidly verify the food state.

**CONCLUSIONS**

In summary, we developed a photochromic composite through a straightforward impregnation method that allowed the incorporation of a functional polymer with appended SP moieties within an FP microfibrous network. The homogenous distribution of the SP-PHEMA polymer around each individual fiber imparted self-similarity properties that preserved the materials’ functionalities upon mechanical scratching and contributed to increase the thermal stability of FP by 12–14 °C, owing to the interactions between the SP-PHEMA polymer and cellulose. Most importantly, a rapid isomerization between the SP and MC forms was registered through spectral and chromic changes in the material upon UV and visible irradiation for 90 and 30 s, respectively, with a significant enhancement compared with the kinetics of photoconversion measured for the SP-PHEMA polymer deposited on glass, for which 150 and 40 s of UV and vis irradiation were required, respectively. The faster photochromism, coupled to an unprecedented reversibility for more than 100 photoswitching cycles, was attributed to the higher conformational flexibility of the SP moieties embedded in the SP-PHEMA chains upon dispersion in the cellulose matrix. In addition, we exploited the photoactivated acidochromism of SPs to demonstrate the potential utilization of the composite for smart food packaging.
applications. By monitoring samples of milk and red wine for 10 days using FP/SP-PHEMA caps, the changes in the chemical environment (pH and total acidity), ascribed to the acidic byproducts of the microbial activity and of deterioration processes, could be well correlated to the response of the composite films. In particular, the system was even sensitive to variations in the acidity of wine that generated changes of just 1% in the pH and of 2.5% in the total acidity, possibly indicative of spoilage processes. Specifically, the acidic vapors generated during the samples deterioration could be detected optically, through the blue shift in the absorption maximum of the photogenerated MC upon protonation to its MCH counterpart, and visually by naked eye, through the progressive transition of the substrates from pink, characteristic of MC, to pale yellow, characteristic of MCH.

Owing to its outstanding photochromic performance and the acidochromic detection in combination with the low-cost and renewability of the paper substrate, the developed material constitutes ideal smart chips and caps for smart packaging that would allow the in situ assessment of the shelf life of food products against counterfeiting and microbial contaminations in both quality control operations and at the consumer end. Moreover, it could as well be integrated within various platforms for environmental monitoring and for a wide range of applications in textile and printing technologies.

## METHODS

**Materials.** Tetrahydrofuran (THF), azobisisobutyronitrile (AIBN), 4-dimethylaminopyridine, trimethylamine, Na₂CO₃, diethyl ether, hexane, MgSO₄, methacryloyl chloride, ethyl acetate (EtOAc), 1-(2-hydroxyethyl)-3-dimethyl-6-nitrospiro-(2H-1-benzopyran-2,2-indole) (SP), dichloromethane (CH₂Cl₂), chloroform, fumaric acid (99% v/v), and HCl (37% v/v) were purchased from Sigma-Aldrich. Commercial fat reduced UHT and pasteurized milk and three red wines made mostly from Sangiovese grape, DOCG (N) (13.5% ABV), were obtained from local supermarkets.

**Synthesis of SP Methacrylate (SPMA).** The reaction scheme is shown in the Supporting Information file (Scheme S2). In a Schlenk flask, freshly distilled HEMA (1 g, 7.7 mmol), SPMA (0.323 g, 0.78 mmol), and AIBN (0.1480 mg, 0.0009 mmol) were dissolved in 25 mL of dry THF under nitrogen atmosphere. The solution was subjected to three freeze–vacuum–thaw cycles and subsequently heated in a thermostated oil bath at 65 °C overnight. After that, the reaction mixture was cooled with liquid nitrogen to stop the polymerization, diluted with 5 mL of chloroform, and exposed to air. Solvent and monomer were reduced under vacuum, and the polymerization mixture was purified by precipitation with 250 mL of dry diethyl ether. The weight and number average molecular weights of the synthesized polymer, M₆ and Mₙ, are 19 752 and 14 588 g/mol, respectively. The polydispersity index is 1.3539. A shown in the Supporting Information (Figure S1), the ¹H NMR (500 MHz, CDCl₃, δ in ppm) peaks assigned to the polymer are: 7.94 (m, 2H), 7.43 (m, 2H), 7.15 (d, 1H), 6.93 (d, 1H), 6.88 (d, 1H), 6.78 (d, 1H), 5.80 (d, 1H), 4.27–3.94 (br, 1H), 3.8–3.72 (d, 2H), 3.44–3.38 (d, 2H), 2.56–2.2 (br, 2H), 1.98–1.82 (br, 2H), 1.72–1.42 (br, 3H), 1.31 (s, 6H), 1.20–1.06 (br, 6H), 1.04–0.96 (br, 3H). The number of SP functionalities (M₆ = 351 g/mol) was determined to be 12 per polymer chain through NMR analysis, corresponding to a total amount of 29% mol in the polymer.

**Preparation of FP/SP-PHEMA.** The FP (2 × 2 cm² and approximately 400 μm thick) was impregnated through immersion in a 3% wt and in a 25% wt SP-PHEMA polymer solution in ethanol for 30 s and dried at room temperature. The amount of polymer with respect to FP was determined to be 1 and 17% wt, respectively, through TGA in the conditions reported below. Unless otherwise stated, the results and discussion refer to FP impregnated with 1% wt of SP-PHEMA.

**Characterization.** The ¹H NMR spectrum of SP-PHEMA was recorded at 298 K using a Bruker AV-500C spectrometer. Molecular weights and molecular weight distribution were determined by gel permeation chromatography (GPC) using Viscotek GPC/SEC TDAmax (Malvern, UK). The chromatographic system was equipped with a GPC column (TSKgel SuperMultiPE HZ-H), triple detector array (TDA 305) consisting of a differential refractive detector, a light scattering detector and a dual capillary viscometer detector. The GPC chromatography was carried out using THF as a solvent, calibrated with polystyrene standard.

The morphology of FP before and after impregnation with the SP-PHEMA was investigated through an optical microscope (Carl Zeiss MTB2004).

The chemical analysis of the SP-PHEMA deposited on a glass slide, of FP and of the FP/SP-PHEMA composite was performed with a FTIR spectrometer (Equinox 70 FT-IR, Bruker) coupled to an attenuated total reflectance (ATR) accessory (MIRacle ATR, PIKE Technologies). Each spectrum was acquired accumulating 128 scans in the 4000–600 cm⁻¹ spectral range with a scanning resolution of 4 cm⁻¹. In addition, XPS measurements were performed, using a SPECTLαb spectrometer with a non-monochromatic Mg Kα source (hv = 1253.6 eV) operated at 15 kV with an emission current of 10 mA. A charge neutralizer consisting of low-energy (ca. 7 eV) electrons was applied and energy-scale calibration was performed by setting the C–C–C–H component of C 1s spectrum at 285 eV. Spectra deconvolution was carried out using CasaXPS software.

The thermal decomposition of the samples was investigated by means of TGA using a TGA (Q500, TA Instruments).
Measurements were performed on 3–5 mg of samples in an aluminum pan under inert N₂ atmosphere with a flow rate of 50 mL/min, at a temperature range from 30 to 600 °C and at a heating rate of 10 °C·min⁻¹. The weight loss and its first derivative were recorded simultaneously as a function of time/temperature.

Absorption spectra of the SP-PHEMA deposited on glass and of FP/SP-PHEMA were collected through a UV–visible–NIR spectrophotometer ( Cary 6000i;Varian) in transmission mode, fixing each sample on a sample holder with a 1 × 1 cm aperture and using untreated FP and glass slides as references for the baseline correction. The recovery of the samples after UV irradiation was determined through spectral scans in the 300–700 nm region every 10 min for 60 min. Emission spectra and confocal images of the FP/SP-PHEMA composite were acquired through a confocal microscope system (A1, Nikon, Japan). Excitation at 560 nm with an energy density of 2.6 × 10⁻⁴ mJ·µm⁻² on a spot area of 0.12 µm² was performed using the continuous laser unit integrated within the microscope setup and the emission signal in the 580–680 nm range was collected by a 4-PMT 32-channels spectral detector with a resolution of 512 × 512 pixels and a bi-directional scanning speed of 1 fps operated by a Galvano scanner.

To evaluate the scratch resistance of the FP/SP-PHEMA, linear scratches were performed on a micro-combi tester (Anton Paar Gmbh, Germany) equipped with a diamond Rockwell indenter tip of 100 µm radius. The combinations of different scratch loads (0.5, 1.0 and 2.0 N) and scratch events (1, 5, and 10) were evaluated at a constant tip speed of 2 mm·min⁻¹ and scanning length of 2 mm. All of the scratch tests were carried out at laboratory conditions (T = 21 ± 1 °C, 50 ± 5% RH). After the test, the samples were imaged through confocal microscopy using the same parameters described above. Z-Stacks of a 200 µm optically thick section were acquired to determine the optical depth in each frame and to reconstruct each 3D scratch profile.

Irradiation of the Samples. UV irradiation was carried out for 0–180 s using a bromograph chamber (MF 1030/C, Nuova Delta Elettronica s.n.c., Italy), equipped with four 15 W UV neon tubes emitting at λ = 320–375 nm with a power density of 4.15 mW·cm⁻². Visible irradiation was conducted in a bromograph chamber (MF 1030/C, Filters) with a power density of 5 mW·cm⁻². The weight loss and its first derivative were recorded simultaneously as a function of time/temperature.

Detection of Acid Vapors. FP/SP-PHEMA samples were inserted in hollow screw caps to seal several vials that contain HCl, water, ethanol, acetaldehyde, ethyl acetate, acetic, or lactic acid. The samples were kept in the dark at room temperature for different times ranging from 60 s to 10 days and were then UV irradiated for 90 s (λ = 320–375 nm, power density = 4.15 mW·cm⁻²) to determine the color and optical changes of the material upon exposure to the vapors of each compound.
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■ DEDICATION
Dedicated to the memory of Carlo Montemagno.

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