JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Photosensitizing Properties of Protein Hydrolysate-Based Fertilizers

LUCIANO CAVANI,*,† ALEXANDRA TER HALLE,‡ CLAIRE RICHARD,‡ AND CLAUDIO CIAVATTA†

Dipartimento di Scienze e Tecnologie Agroambientali, Alma Mater Studiorum−Università di Bologna, Viale Fanin 40, I-40127 Bologna, Italy, and Laboratoire de Photochimie Moléculaire et Macromoléculaire, UMR 6505 CNRS−Université Blaise Pascal, F-63177 Aubière Cedex, France

The use of protein hydrolysate-based fertilizers (PHF) as adjuvant for pesticides or herbicides has been proposed; however, the behaviors of mixtures of PHFs and pesticides under solar light are not known, and various photochemical reactions may occur. The photosensitizing properties of PHFs were investigated in water solutions (0.8 g of total organic carbon $L^{-1}$) within the wavelength range of 300−450 nm, using furfuryl alcohol (FA) as a probe to test the involvement of singlet oxygen and Irgarol 1051 as an example of organic pollutant. Two commercial PHFs and one standard PHF were studied, all of the products being of animal origin. PHFs photosensitize the transformation of FA ($10^{-4}$ M), and the kinetics of FA disappearance follows an apparent first-order rate law. Through the use of sodium azide ($1 \times 10^{-3}$ M) as singlet oxygen scavenger and deuterium oxide ($D_2O$) for increasing the singlet oxygen lifetime it was shown that singlet oxygen contributes largely to the phototransformation of FA. The replacement of water by $D_2O$ increases the apparent first-order rate constant 6 times, whereas the addition of sodium azide reduces it by approximately 90%. These results are confirmed using Irgarol 1051 ($10^{-5}$ M). The photosensitizing properties of PHFs might be due to pigments naturally present in tissues from which they are extracted or to compounds generated during the production processes.

**KEYWORDS: Protein hydrolysate fertilizers; adjuvant; furfuryl alcohol; Irgarol 1051; singlet oxygen**

## INTRODUCTION

The protein hydrolysate-based fertilizers (PHFs) are products obtained by chemical, enzymatic, or thermal hydrolysis of many kinds of organic matrices of vegetal and animal origin. The PHFs are currently used, mainly for foliar application, to stimulate plant metabolism (*1*−*3*). Their activities on plants include the increase of stress resistance (i.e., chill, drought, etc.) and an unspecific hormone-like activity on plant metabolism (*4*−*6*). However, at this time the active molecules present in the PHFs, such as amino acids and small peptides, and the metabolic pathway target in the plants are unknown.

Some authors (*7*−*9*) have proposed the use of PHFs in plant nutrition as chelating agents of different cations (i.e., $Fe^{2+}$, $Ca^{2+}$) for the cure of plant deficiencies. More recently, some PHF-producing companies have proposed to employ the PHFs as adjuvants (or surfactants) for pesticides or herbicides, claiming that these products are able to enhance their activity. Several explanations have been proposed to support this use: the PHFs could increase (i) the solubility of the active ingredients (ai) of the pesticides; (ii) the adhesion and the retention of the ai on the target surfaces; (iii) the uptake and the translocation of the ai in the plant tissues; and (iv) the tolerance of the plants to the ai, reducing the phytotoxicity; and could decrease (v) the photodegradation of the ai on the leaves' surfaces. It is possible that the positive effect of the PHFs as adjuvant is due to their capacity to protect the ai from the naturally occurring photodegradation through a screen effect, but, earlier studies have also shown that several adjuvants are able to induce the photodegradation of different kinds of ai (*10*−*13*).

Due to the presence of reactive chemical groups, PHFs are potentially able to interact with the ai through several mechanisms such as adsorption, solubilization, catalysis, photosensitization, and others. Before they are employed in mixtures with ai, it is important to get a better insight into their possible interactions and to evaluate their potential environmental impact. Until now, though, little is known, especially from the photochemical point of view. In agricultural practices, PHFs are sprayed on the plants together with the ai. Thus, the leaf's surface is their first environment after application. Then, the leaching of PHFs should occur, and PHFs will go onto the soil or into the aquatic environment, where they will be mixed with other constituents of natural waters that are known to exhibit photoinductive or photosensitizing properties such as nitrate ions that photogenerate hydroxyl radicals (*14*) or dissolved organic

* Corresponding author (telephone +39 051 2096209; fax +39 051 2096203; e-mail lcavani@agrsci.unibo.it).
† Università di Bologna.
‡ Université Blaise Pascal.

10.1021/jf0624953 CCC: $33.50 
Published on Web 10/28/2006

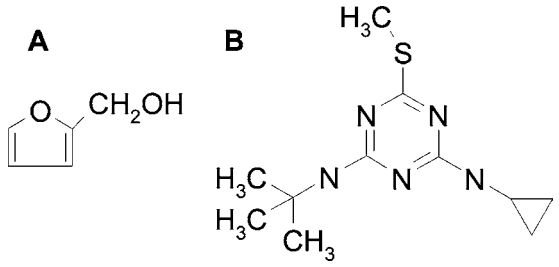


**Figure 1.** Structures of furfuryl alcohol (**A**) and Irgarol 1051 (**B**).

matter that are able to produce a variety of reactive species under solar light absorption (*15*). On the leaves as in the aquatic medium, the ability of PHFs to produce reactive species is of importance for the degradation of organic pollutants.

In a first step, we focused the study on singlet oxygen. This species is produced by energy transfer from triplet excited state of sensitizer to ground state oxygen according to

$$\text{sensitizer}^* + {}^3O_2 \rightarrow \text{sensitizer} + {}^1O_2$$

If conditions for the transfer are fulfilled, this reaction should occur in liquid medium as in solid state. To investigate the ability of PHFs to sensitize the singlet oxygen production, we used furfuryl alcohol (FA) as a probe molecule and chose water as a medium because different kinds of tests can be put in work to ensure the involvement of singlet oxygen (addition of specific scavengers, isotopic $H_2O-D_2O$ effect). The pesticide Irgarol 1051 was also tested to validate results gained with furfuryl alcohol.

## MATERIALS AND METHODS

**Reagents.** A reagent grade of furfuryl alcohol (2-hydroxymethylfuran, **Figure 1**) and sodium azide ($NaN_3$) was obtained from Aldrich (Milwaukee, WI); 2-propanol (iPrOH) and deuterium oxide ($D_2O$) were obtained from Fluka (Buchs, Switzerland). The pesticide Irgarol 1051 (2-methylthio-4-terbutylamino-6-cyclopropylamino-*s*-triazine, **Figure 1**) was purchased from Riedel-de-Haen and used as received.

**Samples.** In this study we have used two commercial PHFs purchased on the market (PHF01 and PHF02) and one "standard" PHF obtained from Fluka: peptone from gelatin, pancreatic digest (PG). The main chemical characteristics of the samples are reported in **Table 1**. The aqueous solutions of PHFs and PG are not very stable because of possible bacterial growth, so they were used immediately after being made and were not kept for >2 days. The addition of sodium azide ($10^{-3}$ M) slightly increased the PHF absorption spectra below 270 nm.

**PHFs Characterization.** Moisture was determined by weight loss at 105 °C; ash by residue on ignition at 550 °C; organic matter (OM) by loss on ignition (OM = dry matter − ash); pH in water (3/50, w/v); electrical conductivity (EC) in water (1/10, w/v); total organic carbon (TOC) by wet oxidation method with potassium dichromate; total nitrogen (TKN) with Kjeldahl method; ammonium nitrogen ($NH_4^+$-N) by extraction with diluted HCl and steam distillation with magnesium oxide; and total organic nitrogen (TON) by difference (TON = TKN − $NH_4^+$-N). Total phosphorus, sulfur, and metals were determined by acid digestion with ultrapure nitric acid (Merck, Darmstadt, Germany) and determination by induced coupled plasma atomic emission spectroscopy (Spectro Ciros$^{CCD}$, Kleve, Germany). The total amino acids (TAA) were determined by RP-HPLC after 23 h of hydrolysis using 6 M HCl and derivatization with 9-fluorenylmethoxycarbonyl chloride (FMOC, Fluka) (*16*). Total tryptophan was determined by RP-HPLC with direct fluorescence detection after basic hydrolysis with barium hydroxide (*17*). Free amino acids (FAA) were extracted using 0.1 M HCl for 1 h and determined by RP-HPLC after derivatization with FMOC (*16*). The degree of hydrolysis (DH) was calculated on the basis of free α-amino nitrogen/total organic nitrogen ratio (*18*). The free α-amino nitrogen was determined with the *o*-phthaldialdehyde (OPA)−*N*-acetylcysteine (NAC) spectrophotometric assay (*19*). The total phenols (TPh) contents were determined with Folin−Ciocalteu's method (*20*). The apparent molecular weights of PHFs was determined by size exclusion chromatography (HP-SEC) using a Bio-Sil SEC 125-5 (Bio-Rad, Hercules, CA) column (300 mm × 7.8 mm) and 0.05 M phosphate buffer + 0.15 M NaCl at pH 6.8 as a mobile phase. PHFs were diluted in the mobile phase at the concentration of 1 g of TOC $L^{-1}$ and filtered on 0.2 $\mu$m filters (Millipore, Bedford, MA) before injection into the column. The chromatograms were recorded at $\lambda = 214$ nm using a UV detector. Weight-average molecular weight (MW) was calculated using the equation

$$\text{MW} = \frac{\sum_i n_i M_i^2}{\sum_i n_i M_i} \quad (1)$$

where $M_i$ and $n_i$ are the molecular weight and the height of each *i*th fraction eluted at the *i*th volume in the chromatogram, respectively (*21*). The UV−visible spectra (220−700 nm) were recorded on a Cary 3 (Varian, Palo Alto, CA) spectrometer. The PHFs were diluted in Milli-Q water at the concentration of 0.8 g of TOC $L^{-1}$, and the spectra were recorded against Milli-Q water in 10 mm length quartz cells.

**Irradiation Equipment.** Irradiation experiments were carried out using a special device equipped with six TLAD 15W05 (Philips, Eindhoven, The Netherlands) fluorescent tubes emitting within the wavelength range of 300−450 nm; these tubes give a broad emission, the maximum of which is located at 365 nm. The Pyrex glass reactor (i.d. = 14 mm) was placed at the center of the device. The cutoff was the following: 5, 34, 66, and 87% of light transmitted at 300, 320, 340, and 360 nm, respectively.

**Photoinductive Experiments.** Solutions of furfuryl alcohol ($2 \times 10^{-2}$ M) and PHFs (8 g of TOC $L^{-1}$) were diluted in Milli-Q water,

**Table 1.** Characteristics of PHF Samples (All Data Are Expressed on a Dry Matter Basis Excluding Those Marked with an Asterisk)

| property | unit | PG | PHF01 | PHF02 |
|---|---|---|---|---|
| source | | animal (collagen from porcine skin) | animal (connective tissue) | animal (collagen and elastine) |
| density* | kg $dm^{-3}$ | ND[a] | 1.28 | 1.25 |
| moisture* | % | 3.4 | 32.0 | 46.2 |
| ash* | % | 6.19 | 11.8 | 25.8 |
| organic matter* | % | 93.8 | 88.2 | 74.2 |
| pH * | | 7.2 | 6.9 | 6.1 |
| $EC_w$[b] * | dS $m^{-1}$ | 6.4 | 5.1 | 27 |
| TOC[c] | g $kg^{-1}$ | 441 | 434 | 405 |
| TKN[d] | g $kg^{-1}$ | 164 | 132 | 112 |
| $NH_4^+$-N | g $kg^{-1}$ | 3.6 | 19.1 | 3.7 |
| TON[e] | g $kg^{-1}$ | 160 | 113 | 108 |
| C/N ratio | | 2.7 | 3.3 | 3.6 |
| total Na | g $kg^{-1}$ | 29.9 | 53.9 | 152 |
| total S | g $kg^{-1}$ | 11.0 | 10.0 | 36.2 |
| total K | g $kg^{-1}$ | 0.345 | 1.50 | 13.6 |
| total Ca | g $kg^{-1}$ | 0.681 | 2.03 | 0.874 |
| total Mg | g $kg^{-1}$ | 0.052 | 0.274 | 0.712 |
| total P | mg $kg^{-1}$ | 474 | 4.60 | 3.30 |
| total Fe | mg $kg^{-1}$ | 2.66 | 4.88 | 195 |
| total Al | mg $kg^{-1}$ | 5.38 | 10.2 | 11.5 |
| total Si | mg $kg^{-1}$ | 3.57 | 2.06 | 7.49 |
| total Zn | mg $kg^{-1}$ | 2.93 | 3.18 | 23.6 |
| total Cu | mg $kg^{-1}$ | 1.20 | 1.03 | 9.70 |
| total Mn | mg $kg^{-1}$ | 0.52 | 1.01 | 7.04 |
| total Ni | mg $kg^{-1}$ | 0.84 | 3.03 | 4.09 |
| total Pb | mg $kg^{-1}$ | 1.43 | 1.84 | 3.22 |
| total Cr | mg $kg^{-1}$ | 1.27 | 1.42 | 1.70 |
| total Cd | mg $kg^{-1}$ | 0.52 | 0.74 | 0.92 |

[a] ND, not determined. [b] $EC_W$, electrical conductivity. [c] TOC, total organic carbon. [d] TKN, total Kjeldahl nitrogen. [e] TON, total organic nitrogen.

**Table 2.** Total Amino Acids (TAA) and Free Amino Acids (FAA) Contents of the PHF Samples (Grams per 100 g of Sample; Data Expressed on a Dry Matter Basis)

| | PG | | PHF01 | | PHF02 | |
|---|---|---|---|---|---|---|
| amino acid | TAA | FAA | TAA | FAA | TAA | FAA |
| Ala | 6.11 | 0.52 | 10.3 | 2.65 | 6.17 | 1.49 |
| Arg | 6.83 | 2.80 | 0.78 | 0.03 | 3.55 | 0.04 |
| Asp + Asn | 6.12 | 0.10 | 4.19 | 0.88 | 4.35 | 0.37 |
| Cys | 0.62 | 0.10 | ND[a] | <0.01[b] | 1.20 | <0.01[b] |
| Glu + Gln | 9.63 | 0.21 | 8.90 | 0.44 | 7.34 | 0.56 |
| Gly | 13.6 | 0.31 | 20.0 | 6.18 | 11.1 | 0.74 |
| His | 3.00 | 0.31 | 1.46 | 0.47 | 0.56 | 0.09 |
| Ile | 1.45 | 0.31 | 1.44 | 0.15 | 1.15 | 0.19 |
| Leu | 3.11 | 1.04 | 2.94 | 0.29 | 2.42 | 0.56 |
| Lys | 3.52 | 0.83 | 2.72 | 0.29 | 2.17 | 0.37 |
| Met | 1.14 | 0.10 | ND | 0.05 | 0.44 | 0.19 |
| Hyp[c] | 10.0 | 0.63 | 6.94 | 1.39 | 6.06 | 0.11 |
| Phe | 1.97 | 0.83 | 1.90 | 0.15 | 1.34 | 0.19 |
| Pro | 10.6 | 0.10 | 11.6 | 2.21 | 7.38 | 0.93 |
| Ser | 3.31 | 0.10 | 0.53 | 0.29 | 2.04 | 0.56 |
| Thr | 1.76 | 0.21 | <0.01[b] | <0.01[b] | <0.01[b] | <0.01[b] |
| Trp | <0.01[b] | <0.01[b] | 0.13 | <0.01[b] | 0.01 | <0.01[b] |
| Tyr | 0.83 | 0.41 | 0.99 | 0.15 | 0.22 | 0.19 |
| Val | 2.17 | 0.31 | 2.31 | 0.15 | 1.78 | 0.56 |
| $total_{DM}$[d] | 85.7 | 9.22 | 77.1 | 16.2 | 59.3 | 7.11 |
| $total_{IS}$[e] | 0.165 | 0.018 | 0.209 | 0.044 | 0.217 | 0.026 |

[a] ND, not determined. [b] Limit of quantification. [c] Hydroxyproline. [d] Total in dry matter. [e] Total in irradiated solutions.

and the two solutions were mixed just before the irradiation and poured into the reactor (50 mL). The final concentrations were $1 \times 10^{-4}$ M for FA and 0.8 g of TOC $L^{-1}$ for PHFs. Aliquots (0.5 mL) were sampled at selected intervals, and the disappearance of furfuryl alcohol was monitored by HPLC (*22*), using a 4.6 mm × 250 mm Chromsep Omnispher 5 $C_{18}$ column (Varian), a water/methanol mixture (85:15, v/v) as a mobile phase at constant flow of 1 mL $min^{-1}$, and UV detection set at 220 nm. Dark control experiments were carried out for each experiment: no reaction was observed.

Photolysis rate was analyzed using an apparent first-order kinetic equation

$$\frac{dc}{dt} = -kc \rightarrow \ln\frac{c_0}{c} = kt \qquad (2)$$

where $c$ is the concentration of the FA (mol $L^{-1}$), $c_0$ the concentration of the FA (mol $L^{-1}$) at time zero, $t$ the irradiation time (h), and $k$ the apparent first-order rate constant ($h^{-1}$). The $k$ value, the half-life time ($t_{1/2}$), and the uncertainty were calculated from the regression curve using Origin 7 SR2 software (OriginLab Corp., Northampton, MA).

## RESULTS

**PHF Characterization.** The samples were under different physical status: samples PHF01 and PHF02 were in concentrated aqueous solutions with density >1.2 kg $dm^{-3}$ (**Table 1**), whereas sample PG was in freeze-dried form. The main chemical characteristics of the samples are shown in **Table 1**.

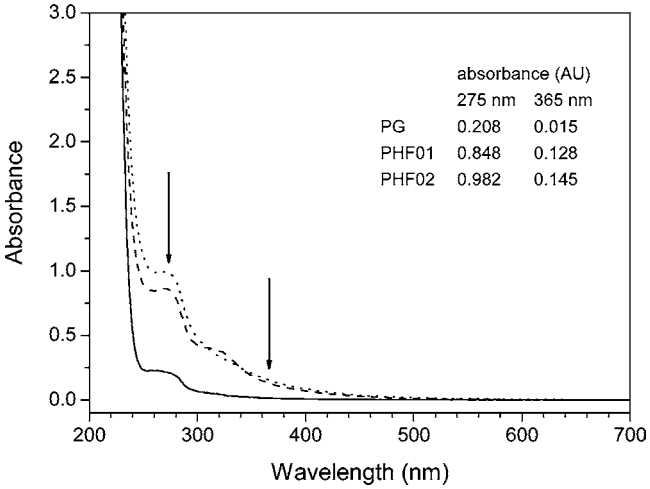


**Figure 2.** UV–vis spectra of PHFs (0.8 g of TOC $L^{-1}$) in Milli-Q water: (—) PG; (- - -) PHF01; (• • •) PHF02.

The water content was higher in PHF02 than in PHF01. PG and PHF01 showed a higher organic matter content than PHF02 that is inversely correlated to the ash content. Compared to the other samples PHF02 had a high content in total sodium, potassium, and sulfur (probably used in the production process). The total amino acids (TAA) content was higher in PG and PHF01 than in PHF02 (**Table 2**), whereas the free amino acids (FAA) content of PHF01 sample was 2 times higher than those found in the other samples (**Table 2**). According to this result, both the free α-amino nitrogen (α-$NH_2$-N) content and the degree of hydrolysis (DH) of PHF01 were high compared to the other samples (**Table 3**). These findings indicate that the samples have been made using different hydrolytic processes and those used in PHF01 production were more effective than the others. The weight-average molecular weight (MW) of PHFs was lower in PHF01 than in PG and PHF02 (**Table 3**). These results confirm that the hydrolytic process employed in PHF01 production was more effective in both FAA releasing and MW reduction. The distribution of the MW shifted toward the fraction of <5 kDa: 63% in PHF01, 25% in PG, and 35% in PHF02 samples (**Table 3**).

The UV−visible spectra of PHFs were recorded at the same TOC concentration (0.8 g of TOC $L^{-1}$) for a better comparison of the different samples. This concentration is the same as that used for the photochemical experiments. The UV−visible spectra (**Figure 2**) showed a high absorbance at $\lambda$ < 240 nm (generally, the polypeptides show the maximum absorbance at 214−220 nm) and two shoulders: the first at 270−280 nm (probably due to the aromatic compounds) and the second one at 320 nm. The intensity of absorbance at 275 and 365 nm was correlated to the total phenols (TPh) content (**Figure 2**; **Table 3**). The absorption was recorded up to 500 nm in the case of

**Table 3.** Free α-Amino Nitrogen (α-$NH_2$-N), Degree of Hydrolysis (DH), Weight-Average Molecular Weight (MW), Molecular Weight Distribution, and Total Phenols (TPh) of PHF Samples

| | α-$NH_2$-N[a] | | | | distribution[b] (%) | | | TPh[e] | |
|---|---|---|---|---|---|---|---|---|---|
| sample | IS[c] ($\mu$g of N $g^{-1}$) | DM[d] (mg of N $g^{-1}$) | DH (%) | MW[a] (kDa) | <5 kDa | 5–10 kDa | >10 kDa | IS[c] ($\mu$g $g^{-1}$) | DM[d] (mg $g^{-1}$) |
| PG | 39.8 (0.2) | 21.3 (0.1) | 13.2 | 12.6 (0.03) | 25.1 | 32.2 | 42.7 | 8.54 (0.08) | 4.58 (0.04) |
| PHF01 | 84.1 (1.1) | 35.3 (0.6) | 34.0 | 8.78 (0.05) | 62.9 | 25.5 | 11.7 | 19.6 (0.1) | 10.6 (0.1) |
| PHF02 | 43.3 (0.4) | 15.7 (0.2) | 14.9 | 13.9 (0.01) | 35.5 | 29.2 | 35.3 | 26.3 (0.6) | 13.3 (0.3) |

[a] In parentheses are reported the standard errors of the mean ($n = 3$). [b] Expressed as percent of the total area of the chromatogram. [c] IS, irradiated solutions. [d] DM, dry matter. [e] Expressed as gallic acid; in parentheses are reported the standard errors of the mean ($n = 3$).

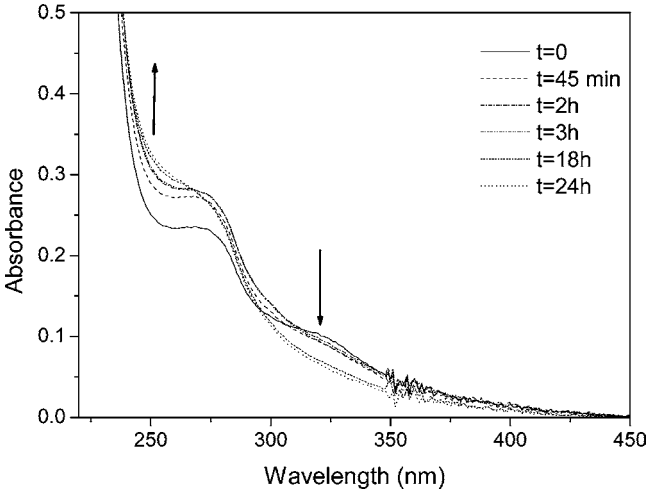


**Figure 3.** Photobleaching of PHF01 (0.8 g of TOC $L^{-1}$) when irradiated with polychromatic light (300–450 nm).

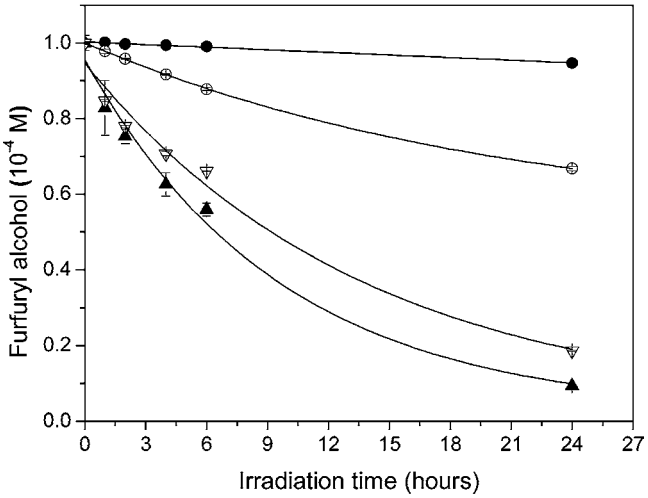


**Figure 4.** Kinetics of furfuryl alcohol ($10^{-4}$ M) photodegradation in polychromatic light (300–450 nm): (●) alone (control); (○) in the presence of PG; (▲) in the presence of PHF01; (▽) in the presence of PHF02. All PHFs were added at 0.8 g of TOC $L^{-1}$

.

PHF01 and PHF02 and up to 400 nm in the case of PG. Within the wavelength range of 250−400 nm the sample PG has shown less absorption than PHF01 and PHF02 samples.

**Irradiation of PHF Alone.** PHF01 (0.8 g $L^{-1}$) was irradiated in pure water during 24 h. After only 45 min of irradiation, the absorption spectrum changed: the shoulder with maximum around 325 nm was significantly reduced, whereas the absorbance below 300 nm increased (**Figure 3**). Between 45 min and 3 h changes were small. The decrease of absorbance was more pronounced after 18 and 24 h of irradiation, leading to a complete disappearance of the 325-nm shoulder.

**Photosensitized Transformation of Furfuryl Alcohol.** The photosensitizing properties of the PHFs were studied using furfuryl alcohol as a probe. FA is a compound relatively stable under light irradiation, but it is degraded in the presence of photosensitizers (*23*). In particular, FA shows a specific reactivity toward singlet oxygen ($^1O_2$*) and hydroxyl radicals (OH•), and the main products of photodegradation of FA are well-known (*23*). Therefore, this alcohol is a very suitable probe to investigate the photosensitizing properties of PHFs.

The photochemical experiments were carried out in water solutions of PHFs prepared at the same TOC concentration (0.8 g $L^{-1}$). The concentration of 0.8 g of TOC $L^{-1}$ was chosen in agreement with that suggested by the manufacturers; it corresponds to 3−4 g $L^{-1}$ of PHFs.

When irradiated alone within the range of 300−450 nm, FA ($10^{-4}$ M) was transformed by <5% after 24 h of exposure (**Figure 4**). In the presence of PHF01 and PHF02 (0.8 g of TOC $L^{-1}$) approximately 80% of FA was transformed, whereas in the presence of PG sample the transformation decreased to about 30% (**Figure 4**). The kinetics of FA phototransformation followed a first-order kinetics. First-order rate constants are reported in **Table 4**. All PHFs increased significantly the rate of FA consumption, the rate constants being 4−6-fold higher for industrial PHFs than for the standard sample (PG).

2-Propanol (iPrOH) was added ($5 \times 10^{-3}$ M) to the irradiated solution to scavenge OH• free radicals ($k = 1.9 \times 10^9$ $M^{-1}$ $s^{-1}$) (*24*). After 24 h of irradiation, the PG-mediated photodegradation of FA was inhibited by 15%, whereas the PHF01-mediated photodegradation of FA was not significantly affected (**Figure 5**), indicating that hydroxyl radicals were not involved in FA degradation in the presence of PHF01 and PHF02 and contributed a little to the transformation of FA in the presence of PG.

**Table 4.** Apparent First-Order Rate Constant ($k$) and Half-Life ($t_{1/2}$) of Furfuryl Alcohol ($10^{-4}$ M) When Irradiated between 300 and 450 nm in the Presence of PHF (0.8 g of TOC $L^{-1}$), Sodium Azide ($NaN_3$, $1 \times 10^{-3}$ M), and 2-Propanol (iPrOH, $5 \times 10^{-3}$ M) in Water ($H_2O$) or Deuterium Oxide ($D_2O$) Solutions

| sample | $k$ [a] ($h^{-1}$) | $R^2$ ($n = 6$) | $t_{1/2}$ (h) |
|---|---|---|---|
| FA alone ($H_2O$) | 0.0022 ± 0.0001 | 0.9926**[b] | 314[c] |
| PG ($H_2O$) | 0.0172 ± 0.0006 | 0.9942** | 40.2[c] |
| PG + iPrOH ($H_2O$) | 0.0145 ± 0.0002 | 0.9992** | 48.0[c] |
| PG + $NaN_3$ ($H_2O$) | 0.0098 ± 0.0002 | 0.9978** | 70.6[c] |
| PHF01 ($H_2O$) | 0.0994 ± 0.0026 | 0.9976** | 6.97 |
| PHF01 + iPrOH ($H_2O$) | 0.0918 ± 0.0052 | 0.9893** | 7.55 |
| PHF01 + $NaN_3$ ($H_2O$) | 0.0361 ± 0.0003 | 0.9994** | 19.2 |
| PHF02 ($H_2O$) | 0.0704 ± 0.0026 | 0.9933** | 9.84 |
| PHF02 + iPrOH ($H_2O$) | 0.0749 ± 0.0019 | 0.9963** | 9.26 |
| PHF02 + $NaN_3$ ($H_2O$) | 0.0338 ± 0.0015 | 0.9947** | 20.5 |
| PHF01 ($D_2O$)[c] | 0.5941 ± 0.0139 | 0.9960** | 1.17 |
| PHF01 + $1 \times 10^{-3}$ M $NaN_3$ ($D_2O$) | 0.0718 ± 0.0026 | 0.9961** | 9.65 |
| PHF01 + $2 \times 10^{-3}$ M $NaN_3$ ($D_2O$) | 0.0568 ± 0.0021 | 0.9910** | 12.2 |

[a] Mean ± standard error ($P < 0.01$). [b] **, $P < 0.01$. [c] Extrapolated data.

For singlet oxygen ($^1O_2$*) quenching, the phototransformation of FA was carried out in the presence of sodium azide ($NaN_3$) ($k = 7.9 \times 10^8$ $M^{-1}$ $s^{-1}$) (*25*). $NaN_3$ ($1 \times 10^{-3}$ M) reduced the rate of FA degradation (**Figure 5**). The kinetics of consumption followed an apparent first-order kinetic (**Table 4**), and the constant rate reductions ranged between 43% for PG and 64% for PHF01. These results suggest that singlet oxygen is involved in the FA transformation in the presence of all tested PHFs.

To confirm this, we repeated the photochemical experiments (only for the PHF01 sample) in deuterium oxide ($D_2O$) solutions, because $D_2O$ reduces the $^1O_2$* decay: the decay constant ($k_d$) of $^1O_2$* is $2.5 \times 10^5$ $s^{-1}$ in water against $3.0 \times 10^4$ $s^{-1}$ in $D_2O$ (*24*, *25*). In **Figure 6** is reported the comparison between the kinetics of phototransformation of FA in the presence of PHF01 in water and $D_2O$ solutions. The rate of FA transformation was much higher in $D_2O$ than in $H_2O$: after 3 h, 80% of FA was consumed and the constant rate was 6-fold higher in $D_2O$ than in water (**Table 4**). The contribution of $^1O_2$* on FA transformation could be further confirmed by adding $NaN_3$ ($1 \times 10^{-3}$ and $2 \times 10^{-3}$ M). In the presence of $NaN_3$ the rate of FA

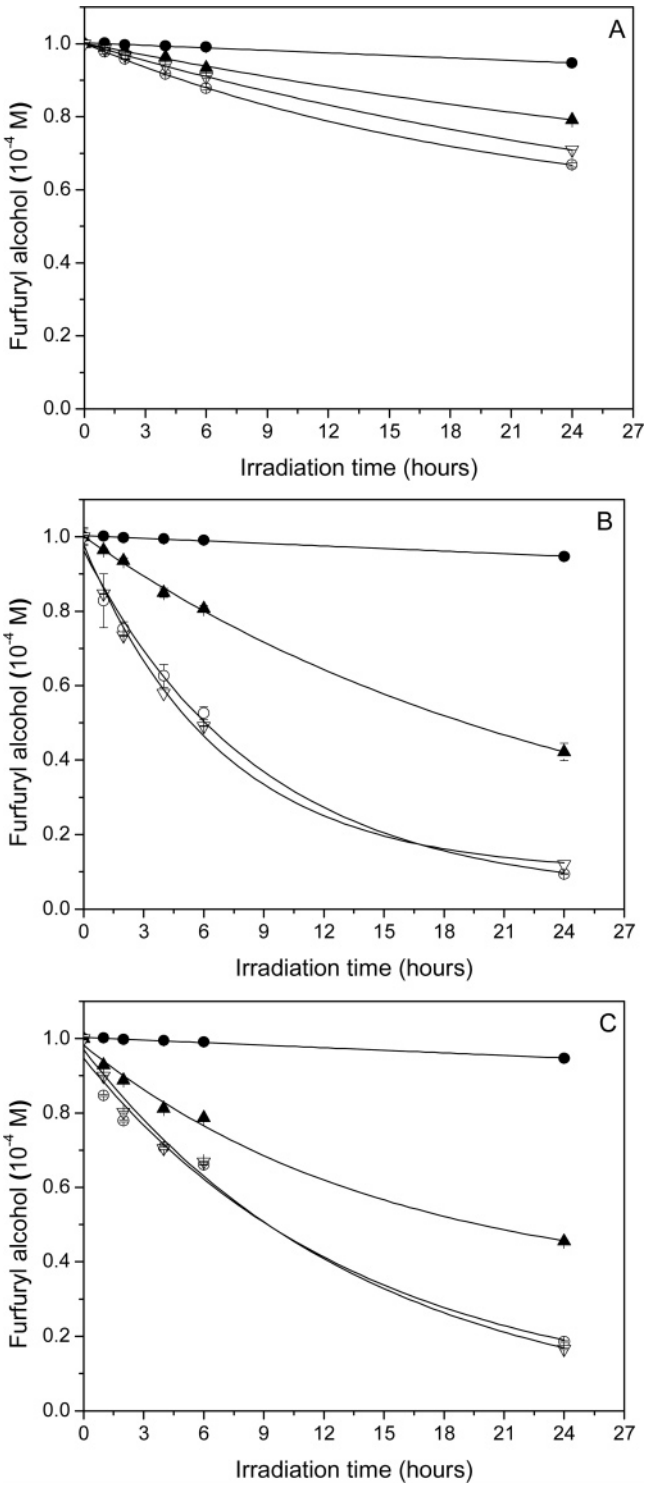


**Figure 5.** Kinetics of furfuryl alcohol ($10^{-4}$ M) photodegradation in polychromatic light (300–450 nm): (●, **A**) alone (control); (○, **A**) in the presence of PG; (▲, **A**) in the presence of PG and sodium azide; (▽, **A**) in the presence of PG and 2-propanol; (●, **B**) alone (control); (○, **B**) in the presence of PHF01; (▲, **B**) in the presence of PHF01 and sodium azide; (▽, **B**) in the presence of PHF01 and 2-propanol; (●, **C**) alone (control); (○, **C**) in the presence of PHF02; (▲, **C**) in the presence of PHF02 and sodium azide; (▽, **C**) in the presence of PHF02 and 2-propanol. All PHFs were added at 0.8 g of TOC $L^{-1}$, sodium azide was added at $1 \times 10^{-3}$ M, and 2-propanol was added at $5 \times 10^{-3}$ M.

transformation in $D_2O$ solutions of PHF01 was reduced approximately by 90% (**Table 4**).

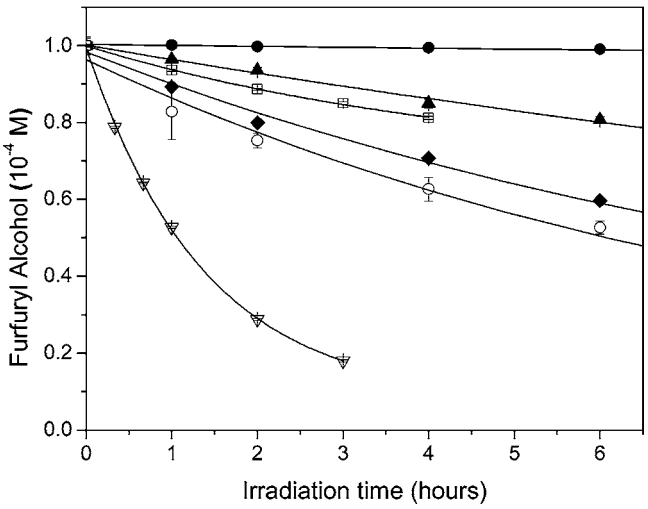


**Figure 6.** Kinetics of furfuryl alcohol photodegradation in polychromatic light (300–450 nm): (●) alone (control); (○) in the presence of PHF01 in $H_2O$; (▲) in the presence of PHF01 and sodium azide ($1 \times 10^{-3}$ M) in $H_2O$; (▽) in the presence of PHF01 in $D_2O$ solution; (◆) in the presence of PHF01 and sodium azide ($1 \times 10^{-3}$ M) in $D_2O$ solution; (□) in the presence of PHF01 and sodium azide ($2 \times 10^{-3}$ M) in $D_2O$ solution. PHF01 was added at 0.8 g of TOC $L^{-1}$.

On the basis of a simple mechanism and using known rate constant values, it is possible to compute inhibition coefficients. If we assume the simple mechanism

$$\mathrm{PHF} + h\nu \rightarrow \mathrm{PHF}^*$$

$$\mathrm{PHF}^* + \mathrm{O_2} \rightarrow \mathrm{PHF} + {}^1\mathrm{O_2}^*$$

$${}^1\mathrm{O_2}^* \rightarrow {}^3\mathrm{O_2} \quad (k_d = 2.5 \times 10^5\ \mathrm{s^{-1}}\ \text{in}\ \mathrm{H_2O}\ \text{and}\ 3 \times 10^4\ \mathrm{s^{-1}}\ \text{in}\ \mathrm{D_2O})$$

$${}^1\mathrm{O_2}^* + \mathrm{FA} \rightarrow \text{products} \qquad (k_1 = 1.2 \times 10^8\ \mathrm{M^{-1}\ s^{-1}})$$

$${}^1\mathrm{O_2}^* + \mathrm{N_3^-} \rightarrow {}^3\mathrm{O_2} \qquad (k_2 = 7.9 \times 10^8\ \mathrm{M^{-1}\ s^{-1}})$$

the rate of singlet oxygen formation, $r$, is equal to $\sum_{\lambda_1}^{\lambda_2} I_a(\lambda)\Phi_T^{O_2}(\lambda)$, where $I_a(\lambda)$ is the light intensity absorbed by PHF at the wavelength $\lambda$, $\Phi_T^{O_2}(\lambda)$ is the apparent quantum yield of singlet oxygen formation at $\lambda$, and $\lambda_1$ and $\lambda_2$ are the spectral range for PHF absorption.

The rate of FA disappearance in irradiated solutions of PHF can be described by the following rate law:

$$-\frac{\mathrm{d[FA]}}{\mathrm{d}t} = r\frac{k_1[FA]}{k_d + k_1[\mathrm{FA}]} \tag{3}$$

In the presence of azide anions ($N_3^-$) the rate law is

$$-\frac{\mathrm{d[FA]}}{\mathrm{d}t} = r\frac{k_1[\mathrm{FA}]}{k_d + k_1[\mathrm{FA}] + k_2[\mathrm{N_3^-}]} \tag{4}$$

FA ($10^{-4}$ M) should trap 4.6% of singlet oxygen in $H_2O$ and 29% in $D_2O$. Experimentally in the case of PFH01 we found a reaction rate constant 6-fold higher in $D_2O$ than in $H_2O$, in excellent agreement. $NaN_3$ ($10^{-3}$ M) should reduce the rate by 75% in $H_2O$ and by 95% in $D_2O$. Actually, reductions were equal to 64 and 88%, respectively, in a good accordance, too.

**Photosensitized Transformation of Irgarol 1051.** The capacity of PHFs to photosensitize the transformation of

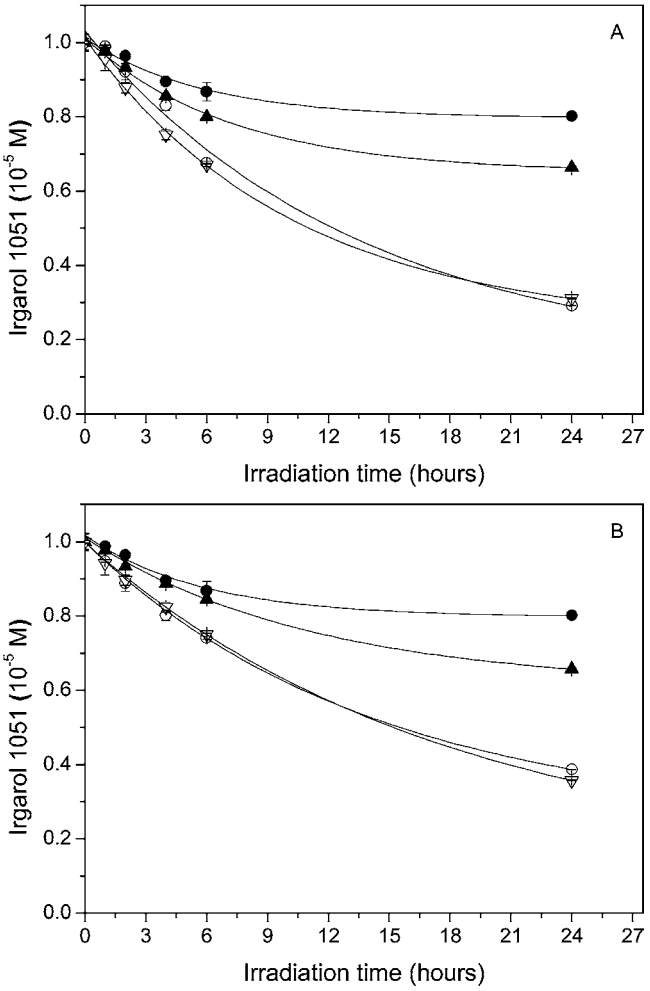


**Figure 7.** Kinetics of Irgarol 1051 photodegradation in polychromatic light (300–450 nm): (●, **A**) alone (control); (○, **A**) in the presence of PHF01; (▲, **A**) in the presence of PHF01 and sodium azide; (▽, **A**) in the presence of PHF01 and 2-propanol; (●, **B**) alone (control); (○, **B**) in the presence of PHF02; (▲, **B**) in the presence of PHF02 and sodium azide; (▽, **B**) in the presence of PHF02 and 2-propanol. PHFs was added at 0.8 g of TOC $L^{-1}$, sodium azide was added at $1 \times 10^{-3}$ M, and 2-propanol was added at $5 \times 10^{-3}$ M.

pesticide was finally tested using Irgarol 1051, a herbicide used as an antifouling agent. This compound that bears an S atom (**Figure 1**) was previously found to be oxidized when irradiated in the presence of humic substances (*26*). **Figure 7** gives the results obtained when Irgarol 1051 ($10^{-5}$ M) was irradiated in the presence of PHF01 and PHF02 (0.8 g of TOC $L^{-1}$). Irgarol 1051 disappeared by 80% after 24 h of irradiation with PHF01 and by 70% with PHF02 (**Table 5**). In contrast, when irradiated alone, the consumption was only equal to 20%. Thus, there is a photoinductive effect of PHFs. As observed in the case of FA, the reaction is not affected by 2-propanol ($5 \times 10^{-3}$ M) but is significantly slowed by sodium azide ($10^{-3}$ M), confirming the involvement of singlet oxygen.

## DISCUSSION

The results reported here give some information on the photochemical behavior of PHFs when irradiated within the wavelength range of 300−450 nm. First, they undergo photobleaching that suppresses the shoulder at 325 nm and increases the absorbance below 300 nm (**Figure 3**). Second, PHFs are able to photoinduce the transformation of FA. Hydroxyl radicals

**Table 5.** Apparent First-Order Rate Constant ($k$) and Half-Life ($t_{1/2}$) of Irgarol 1051 ($10^{-5}$ M) When Irradiated between 300 and 450 nm in the Presence of PHF (0.8 g of TOC $L^{-1}$), Sodium Azide ($NaN_3$, $1 \times 10^{-3}$ M), and 2-Propanol (iPrOH, $5 \times 10^{-3}$ M) in Water Solutions

| sample | $k$ [a] ($h^{-1}$) | $R^2$ ($n = 6$) | $t_{1/2}$ (h) |
|---|---|---|---|
| Irgarol 1051 alone | 0.0105 ± 0.0020 | 0.7945*[b] | 65.8[c] |
| PHF01 | 0.0518 ± 0.0017 | 0.9971** | 13.4 |
| PHF01 + iPrOH | 0.0504 ± 0.0025 | 0.9870** | 13.8 |
| PHF01 + $NaN_3$ | 0.0189 ± 0.0026 | 0.8907* | 36.8[c] |
| PHF02 | 0.0407 ± 0.0017 | 0.9935** | 17.0 |
| PHF02 + iPrOH | 0.0434 ± 0.0008 | 0.9991** | 16.0 |
| PHF02 + $NaN_3$ | 0.0186 ± 0.0015 | 0.9705** | 37.4[c] |

[a] Mean ± standard error ($P < 0.01$). [b] *, $P < 0.05$; **, $P < 0.01$. [c] Extrapolated data.

are generated from irradiated PHFs, but their contribution to FA transformation accounted for <15% at best. We may expect that these very reactive species are efficiently trapped by PHFs, which show good radical scavenging ability (*27*−*29*). The main species involved in FA degradation is singlet oxygen. Its participation in the reaction could be clearly shown using trapping experiments and by replacing $H_2O$ by $D_2O$. PHFs exhibit distinct photosensitizing activities: the commercial products (PHF01 and PHF02) showing higher photosensitizing activity than the standard sample (PG). As the kinetics of FA consumption follows a first order during all of the time of the irradiation, one can conclude that it is not affected by photobleaching. This means that chromophoric constituents of PHFs that undergo photobleaching are not the singlet oxygen producers.

Compared to PG, commercial products are significantly more absorbing at 365 nm (**Figure 2**). Thus, they contain more chromophoric constituents than PG. Even though it is not possible to connect directly absorption and rate of singlet oxygen production, because sensitizing constituents remain unknown, it seems logical to observe the highest rates for the highest absorbing compounds. Detailed examination of chemical data gained for PHFs indicates that DH and MW (**Table 3**) are not correlated with photosensitizing properties; neither are TAA and FAA contents (**Table 2**). The only differences are observed for TPh, which are more concentrated in PHF01 and PHF02 than in PG (**Table 3**), and for some amino acids such as methionine and histidine, which are at higher level in PG than in PHF01 and PHF02 (**Table 2**). The concentration of TPh is quite low (around $10^{-5}$ g/g), and they should not have any impact on the reaction. In contrast, amino acids that are singlet oxygen quenchers, especially methionine ($k = 2.2 \times 10^7$ $M^{-1}$ $s^{-1}$), histidine ($k = 1.5 \times 10^8$ $M^{-1}$ $s^{-1}$), or tyrosine ($k = 2.7 \times 10^7$ $M^{-1}$ $s^{-1}$) (*24*), might scavenge singlet oxygen in PG solutions. PG contains about 3 g of histidine per 100 g of dry matter in bound form. After dilution, it corresponds to a concentration of about 0.3 mM in our samples. The product $k$[histidine] is thus equal to $4.5 \times 10^4$ $s^{-1}$, which is not negligible behind $k_d$. It can be deduced that the lifetime of singlet oxygen is 10−20% shorter in PG solution than in the other PHF solutions. Such a lifetime reduction should contribute to decrease the rate of FA reaction.

The photosensitizing properties of PHFs might be due to pigments such as melanin that are naturally present in tissues from which they are extracted. The industrial production process could also provoke the formation of compounds exhibiting photosensitizing activity. Indeed, the high temperatures could promote the formation of colored compounds toward polymerization or Maillard reactions by condensation of amino acids

with carbohydrates or hydroxylated chemicals. Melanoidins, the brown pigments formed during the Maillard reaction in thermally processed foods, were found to sensitize the production of singlet oxygen (*30*).

PHFs being applied on plants, the question that arises now is whether this photosensitizing effect will exist on leaf surfaces. Further experiments are now needed to evaluate this sensitizing capacity. The sensitizing effect was found in the case of three protein hydrolysate-based fertilizers. It is not possible to extrapolate these data to all, especially because the sensitizer chromophores were not identified, but this property is likely to be common to many of them.

In conclusion, the results obtained in this work have evidenced that tested PHFs exhibit photosensitizing properties and are able under irradiation in the solar spectrum to sensitize the transformation of FA and Irgarol 1051 through the involvement of singlet oxygen. Although PHFs have been characterized, it has not been possible to identify with certainty which are the compounds that are responsible for these photosensitizing properties. The demonstration that these PHFs are able to sensitize the production of singlet oxygen in water solution is not in contradiction with their use as surfactant (*10*). However, it would be now necessary to test them in experimental conditions approaching real ones, in particular, on the surface of leaves.

## ABBREVIATIONS USED

$^1O_2$*, singlet oxygen; DH, degree of hydrolysis; EC, electrical conductivity; FA, furfuryl alcohol; FAA, free amino acids; FMOC, 9-fluorenylmethoxycarbonyl chloride; HP-SEC, high-performance size exclusion chromatography; i.d., inner diameter; $I_a$, light intensity absorbed by PHF; iPrOH, 2-propanol; $k$, first-order rate constant; $k_d$, decay constant of $^1O_2$*; MW, weight-average molecular weight; NAC, *N*-acetylcysteine; $NH_4^+$-N, ammonium nitrogen; OM, organic matter; OPA, *o*-phthaldialdehyde; PG, peptone from gelatin; PHF, protein hydrolysate-based fertilizers; $r$, rate of singlet oxygen formation; RP-HPLC, reverse-phase high-performance liquid chromatography; $t_{1/2}$, half-life time; TAA, total amino acids; TKN, total Kjeldahl nitrogen; TOC, total organic carbon; TON, total organic nitrogen; TPh, total phenols; UV, ultraviolet spectra; α-$NH_2$-N, free α-amino nitrogen; $\lambda$, wavelength; $\Phi_T^{O_2}$, apparent quantum yield of singlet oxygen formation.

## LITERATURE CITED


(1) Kubo, M.; Okajima, J.; Hasumi, F. Isolation and characterization of soybean wastedegrading microorganisms and analysis of fertiliser effects of the degraded product. *Appl. Environ. Microbiol.* **1994**, *60*, 243−247.

(2) Mohiuddin, M.; Das, A. K.; Ghosh, D. C. Growth and productivity of wheat as influenced by integrated use of chemicals fertilizer, biofertilizer and growth regulator. *Indian J. Plant Physiol.* **2000**, *5*, 334−338.

(3) Quartieri, M.; Cavani, L.; Lucchi, A.; Marangoni, B.; Tagliavini, M. Effects of the rate of protein hydrolysis spray concentration on growth of potted kiwifruit (*Actinidia deliciosa*) plants. *Acta Hortic.* **2002**, *594*, 341−347.

(4) Ghosh, D. C.; Nandi, P.; Shivkumar, K. Effect of biofertilizer and growth regulator on growth and productivity of potato (*Solanum tuberosum*) at different fertility levels. *Indian J. Agric. Sci.* **2000**, *70*, 466−468.

(5) Näsholm, T.; Huss-Dannell, K.; Högbberg, P. Uptake of glycine by field grown wheat. *New Phytol.* **2001**, *150*, 59−63.

(6) Chen, S.-K.; Subler, S.; Edwards, C. A. Effects of agricultural biostimulants on soil microbial activity and nitrogen dynamics. *Appl. Soil Ecol.* **2002**, *19*, 249−259.

(7) Hsu, H. H.; Ashmead, H. D.; Graff, D. J. Adsorption and distribution of foliar applied iron by plants. *J. Plant Nutr.* **1982**, *5*, 969−974.

(8) Ashmead, H. D. The absorption mechanism of amino acid chelates by plant cells. In *Foliar Feeding of Plant with Amino Acids Chelates*; Ashmead, H. D., Ashmead, H. H., Miller, G. W., Hsu, H. H., Eds.; Noyes Publications: Park Ridge, NJ, 1986; pp 219−235.

(9) Kalbasi, M.; Shariatmadari, H. Blood powder: a source of iron for the plants. *J. Plant Nutr.* **1993**, *16*, 2213−2223.

(10) Katagi, T. Photodegradation of pesticides on plant and soil surfaces. *Rev. Environ. Contamin. Toxicol.* **2004**, *182*, 1−189.

(11) Tanaka, F. S.; Wien, R. G.; Mansager, E. R. Effect of nonionic surfactant on the photochemistry of 3-(4-chlorophenyl)-1,1-dimethylurea in aqueous solution. *J. Agric. Food Chem.* **1979**, *27*, 774−779.

(12) Tanaka, F. S.; Wien, R. G.; Mansager, E. R. Survey for surfactant effects on the photodegradation of herbicides in aqueous media. *J. Agric. Food Chem.* **1981**, *29*, 227−230.

(13) Thomas, S. M.; Harrison, S. K. Surfactant-altered rates of clorimuron and metsulfuron photolysis in sunlight. *Weed Sci.* **1990**, *38*, 602−606.

(14) Boule, P.; Bolte, M.; Richard, C. Transformations photoinduced in aquatic media by $NO_3^-/NO_2^-$, $Fe^{III}$ and humic substances. In *The Handbook of Environmental Chemistry*; Boule, P., Ed.; Springer-Verlag: Berlin, Germany, 1999; Vol. 2, Part L, pp 181−216.

(15) Richard, C.; Canonica, S. Aquatic phototransformation of organic contaminants induced by coloured dissolved natural organic matter. In *The Handbook of Environmental Chemistry*; Hutzinger, O., Ed.; Springer-Verlag: Berlin, Germany, 2005; Vol. 2, Part M, pp 299−323.

(16) Cunico, R.; Mayer, A. G.; Wehr, C. T.; Sheehan, T. L. High sensitivity amino acids analysis using a novel automated pre-column derivatization system. *Biochromatography* **1986**, *1*, 6−14.

(17) Garcia, S. E.; Baxter, J. H. Determination of tryptophan content in infant formulas and medical nutritionals. *J. AOAC Int.* **1992**, *75*, 1112−1119.

(18) Nielsen, P. M.; Petersen, D.; Dambmann, C. Improved method for determining food protein degree of hydrolysis. *J. Food Sci.* **2001**, *66*, 642−646.

(19) García Alvarez-Coque, M. C.; Medina Hernández, M. J.; Villanueva Camañas, R. M. Studies on the formation and stability of isoindoles derived from amino acids, *o*-phthalaldehyde and *N*-acetyl-L-cysteine. *Anal. Biochem.* **1989**, *180*, 172−176.

(20) Box, J. D. Investigation of the Folin Ciocalteu phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.* **1983**, *17*, 511−525.

(21) Yau, W. W.; Kirkland, J. J.; Bly, D. D. *Modern Size Exclusion Chromatography*; Wiley-Interscience: New York, 1979.

(22) Haag, W. R.; Hoigné, J.; Gassman, E.; Braun, A. M. Singlet oxygen in surface water—part I: furfuryl alcohol as a trapping agent. *Chemosphere* **1984**, *13*, 631−640.

(23) Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. Critical review on rate constants for reactions of hydrated electrons, hydrogen atoms, and hydroxyl radicals in aqueous solution. *J. Phys. Chem. Ref. Data* **1988**, *10*, 809−999.

(24) Wilkinson, F.; Brummer, J. G. Rate constants for the decay and reactions of the lowest electronically excited state of molecular oxygen in solution. *J. Phys. Chem. Ref. Data* **1981**, *17*, 513−886.

(25) Rodgers, M. A. J.; Snowden, P. T. Lifetime of $O_2$ ($^1\Delta_g$) in liquid water as determined by time-resolved infrared luminescence measurement. *J. Am. Chem. Soc.* **1982**, *104*, 5541−5543.

(26) Okamura, H.; Sugiyama, Y. Photosensitized degradation of Irgarol 1051 in water. *Chemosphere* **2004**, *57*, 739−743.

(27) Hattori, M.; Yamaji-Tsukamoto, K.; Kumagai, H.; Feng, Y.; Takahashi, K. Antioxidative activity of soluble elastine peptides. *J. Agric. Food Chem.* **1998**, *46*, 2167−2170.

(28) Kim, S. K.; Kim, Y. Y.; Byun, K. S.; Nam, D. S. Y.; Shahidi, F. Isolation and characterization of antioxidative peptides from gelatine hydrolysates from Alaska Pollack skin. *J. Agric. Food Chem.* **2001**, *49*, 1984−1989.

(29) Wang, L. L.; Xiong, Y. L. Inhibition of lipid oxidation in cooked beef patties by hydrolyzed potato protein is related to its reducing and radical scavenging ability. *J. Agric. Food Chem.* **2005**, *53*, 9186−9192.

(30) Argirova, M. D. Photosensitizer activity of model melanoidins. *J. Agric. Food Chem.* **2005**, *53*, 1210−1214.

**Received for review August 31, 2006. Accepted September 5, 2006. This project was supported with funds provided by the Italian MAP-FIT "BioVeNus" Project (E01/0160) and the Marco Polo Project 2004 of Alma Mater Studiorum University of Bologna.**

JF0624953