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Photosensitizing properties of formulation adjuvants

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ABSTRACT

Protein hydrolysed fertilizers (PHFs) are currently used to stimulate plant metabolism, mainly through foliar application, and are proposed for use as pesticide adjuvants. However, the effects of PHFs on pesticides upon irradiation are unknown. Here, several herbicides and model compounds were irradiated with simulated solar light in the presence of four PHFs extracted from animal proteins and from vegetal. PHFs enhanced the photodegradation of fenuron, mesotrione, 2-mercaptobenzothiazole and 2,4,6-trimethylphenol. Using the scavenging technique, we could evidence the direct reaction of triplet excited states of PHFs towards substrates and we evaluated the contribution of triplets, singlet oxygen and hydroxyl radicals. Hydroxyl radicals were not detected; they were either not formed or were efficiently scavenged by the PHFs. The four PHFs showed distinct photosensitizing abilities. Production processes, rather than origin of the PHFs, seem to govern the photochemical reactivity of the PHFs.

1. Introduction

Protein hydrolysates obtained from many kinds of organic matrices of vegetal or animal origin are employed in agriculture to stimulate plant metabolism [1–5]. These compounds which are claimed to enhance the activity of pesticides are also proposed as adjuvants of active ingredients. Protein hydrolysates-based fertilizers (PHFs) are generally used through foliar application. After having been spread on plants, PHFs are sorbed on the outer leaf layer where they are submitted to solar light. Since PHFs are brown-coloured and light absorbing compounds, their photochemical reactions at the leaf surface is highly probable [6]. In particular, PHFs might produce reactive species under light excitation and sensitize the degradation of pesticides also present on the leaf after crop treatment by spraying.

In a previous study, we have demonstrated that aqueous PHF solutions can sensitize the production of singlet oxygen upon irradiation by simulated solar light [7]. For this, we used furfuryl alcohol as a singlet oxygen scavenger. Since singlet oxygen is a strong oxidant, this primary result suggests that PHFs have the potential to degrade the herbicides they are in contact with [8]. Reactive species other than singlet oxygen may also be produced by irradiation of the PHFs. For instance, triplet excited states, which generate singlet oxygen by energy transfer into ground state oxygen, might also exhibit oxidant properties. The objective of the present work was to assess the photooxidant properties of PHFs more deeply and to focus on oxidant triplet excited states deriving from PHFs. We used a variety of herbicides, as well as model compounds, as substrates [9,10]. The studied compounds are listed in Scheme 1. Reactive species involved in the substrates phototransformation were characterised using the scavenging technique. Three PHFs extracted from animal proteins and one PHF extracted from vegetal were studied.

2. Materials and methods

2.1. Chemicals

Fluometuron (97.7%), mesotrione (99.7%), sulcotrione (98.7%), dichlorprop (99%) and prometryn (>98%) were purchased from Riedel-de-Häen; furfuryl alcohol (99%), 2-mercaptobenzothiazole (98%), fenuron (98%) and 2,4,6-trimethylphenol (99%) were purchased from Aldrich. Sodium azide, 2-propanol and Rose Bengal (RB) were of the highest purity grade available and were used as received. Water was purified using a Milli-Q device (Millipore). Four PHF samples (PHF1, PHF2, PHF3 and PG) were tested. PHF1, PHF2 and PG were obtained from animal protein. The matrices were connective tissue for PHF1 and collagen and elastine for PHF2. PG was a standard sample provided by Fluka and was obtained from gelatin, a polypeptide derived from collagen (the most important protein of animal skin). PHF3 was of vegetal origin and was obtained from alfalfa meal.

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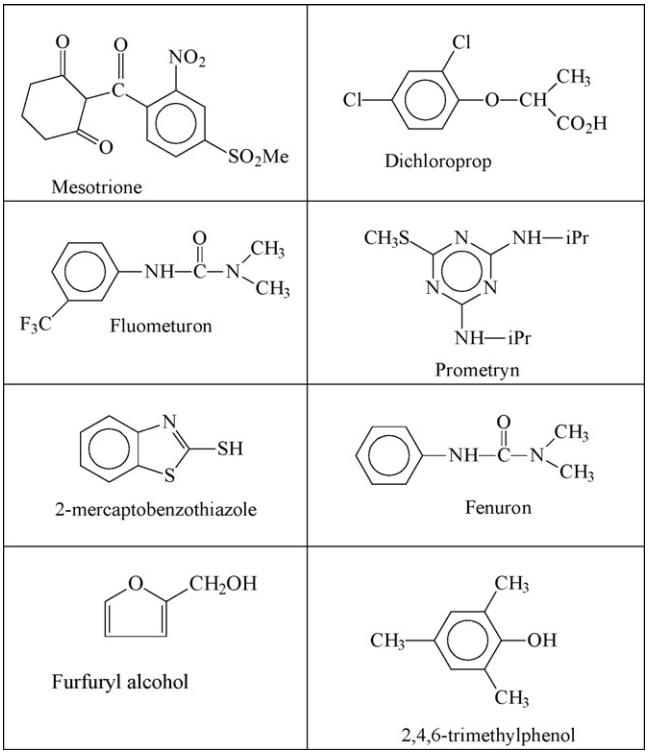

Scheme 1.

2.2. PHFs characterisation

The following characteristics were determined using the methods stated: 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 in water (1/10, w/v); total organic carbon (TOC) by the wet oxidation method with potassium dichromate; total nitrogen with the Kjeldahl method; and total phosphorus and sulphur by acid digestion with ultrapure nitric acid (Merck, Germany) and by induced coupled plasma atomic emission spectroscopy (Spectro Ciros^{CCD}, Germany). The total amino acid content was determined by RP-HPLC after 23 h of hydrolysis using 6 M HCl and derivatisation with 9-fluorenylmethoxycarbonyl chloride (FMOC, Fluka, Switzerland). Free amino acids were extracted using 0.1 M HCl for 1 h and determined by RP-HPLC after derivatisation with FMOC [11]. The degree of hydrolysis (DH) was determined using the relationship: DH (%) = (α-amino-N/organic-N) × 100. The total phenol content was determined with the Folin–Ciocalteu method [12].

2.3. Solution preparation

PHFs were added to Milli-Q purified water to give a solution of 8 g TOC L^{-1} (prepared daily or held for a maximum of 48 h at 4 °C). Stock solutions of the different substrates (10^{-4} M) were prepared independently. They were mixed in proportions to give final solutions of 9×10^{-5} M substrate and 800 mg TOC L^{-1} PHFs. Due to the buffering properties of the PHFs, all solutions showed a pH around 7. Solutions of substrates that did not contain PHFs were buffered at pH 7 using phosphate buffers (10^{-3} M). Solutions containing Rose Bengal (1.7×10^{-5} M) and each of the substrates (9×10^{-5} M) were prepared in Milli-Q water and buffered at pH 6.5 with phosphate buffers (10^{-3} M). The absorbance of the solutions was equal to 0.8 at 546 nm.

2.4. Irradiation devices

The irradiation of the PHF–substrate mixtures was carried out in a device equipped with six TLAD 15W/05 fluorescent tubes emitting a wavelength range 300–450 nm with a maximum emission at 365 nm. The device was cylindrical and was equipped with reflecting inner walls. The Pyrex-glass reactor (14 mm internal diameter) was placed in the centre of the device and surrounded by the six fluorescent tubes. The reactor was filled with 37 ± 2 mL of solution, and cooling was achieved by ventilation. The rate of furfuryl alcohol photodegradation was measured at the beginning and at the end of the set of experiments to evaluate the decrease of the light intensity delivered by the lamps. It was less than 10%. Solutions containing substrates and Rose Bengal were irradiated at 546 nm using a high pressure mercury lamp and a monochromator (Bausch and Lomb). A cut-off filter (420 nm) was placed between the lamp and the samples to ensure that the substrate did not absorb light.

2.5. Irradiation experiments

Solutions containing substrates with or without PHFs were introduced into the Pyrex-glass reactor and irradiated with the polychromatic light. The solutions were aerated during the irradiation by air bubbling. Aliquots of 1 mL were sampled from the solutions at selected irradiation times. They were immediately and directly analyzed by HPLC to monitor the substrate consumption. A part of the solutions containing substrates and PHFs was left in the dark at room temperature during the time of the irradiation experiments to ensure that no dark reactions occurred. The kinetics was generally pseudo-first order: $-d[S]/dt = k_S \cdot t$, where S is the substrate, k_S is the rate coefficient and t is the irradiation time. The values of k_S were determined by plotting $\ln[S]_0/[S]$ *vs.* t and measuring the slope. In the absence of PHFs, the substrate loss rates were proportional to [S] because the substrates poorly absorbed within the wavelength range of the lamp emission. In the presence of PHFs, k_S was the sum of two contributions, one corresponding to direct photolysis and the second to induced reactions involving oxidant species photogenerated by the PHFs.

For the Rose Bengal sensitized photo-oxidation, all substrates were tested in the same experimental conditions. The air-saturated solutions were irradiated in a quartz cell for a time enabling substrate losses within the range 5–10%. The rates of substrate depletion (r_S) were obtained by dividing the substrate losses by the corresponding irradiation times. At low substrate concentration, generally less than 10^{-4} M, the main route of singlet oxygen disappearance was deactivation ($k_d = 2.5 \times 10^5$ s^{-1}) [13]. In these conditions, the rate of substrate loss, r_S, was equal to

$$r_S = r_{SO} \frac{k_S^{OS} \cdot [S]}{k_d} \quad (1)$$

where r_{SO} is the rate of singlet oxygen formation, k_S^{OS} is the bimolecular rate constant of the reaction between singlet oxygen and the substrate and [S] is the substrate concentration. The rate constant of reaction of the substrates with singlet oxygen was estimated using furfuryl alcohol as a reference. The rate constant of reaction of furfuryl alcohol with singlet oxygen is equal to 1.2×10^8 M^{-1} s^{-1} [14]. With the concentration of furfuryl alcohol chosen equal to that of the substrate, the result was

$$k_S^{OS} = 1.2 \times 10^8 \cdot \frac{r_S}{r_{FFA}} \quad (2)$$

where r_{FFA} is the rate of furfuryl alcohol disappearance. In the case of long irradiations, the fact that the Rose Bengal partly disappeared was taken into account. To evaluate the Rose Bengal loss, the absorption at 546 nm was measured before and after irradiation.

Table 1
Main chemical characteristics of PHFs.

Parameters	PG	PHF1	PHF2	PHF3
Water (%)	3.4	32.0	46.2	46.2
Ash (%)[a]	6.19	11.8	25.8	23.0
Organic matter (%)[a]	93.8	88.2	74.2	76.6
pH (1:10 water)	7.2	6.6	5.9	7.0
Electrical conductivity (dS m^{-1})	6.4	5.1	16.0	14.5
Total organic carbon (% C)[a]	44.1	43.4	40.5	36.8
Total nitrogen (% N)[a]	16.4	13.2	11.2	9.4
Organic nitrogen	n.d.	7.7	10.8	
C/N	2.7	3.3	3.6	3.9
Total sulphur (% S)[a]	1.1	1.0	3.6	n.d.
Total phosphorous (mg kg^{-1})[a]	474	4.6	3.3	n.d.
Total amino acids (mg g^{-1})[a]	85.7	77.1	59.3	52.0
Free amino acids (mg g^{-1})	9.2	16.2	7.1	5.4
Total phenols (mmol tyr kg^{-1})	31.4	47.6	47.8	76.3

n.d.: not determined.
[a] Data expressed on dry matter basis.

2.6. Analytical procedures

Substrate losses were monitored by HPLC–UV using a Waters apparatus equipped with two pumps (model 510), an autosampler, a photodiode array detector (model 996) and a C_8 reversed-phase column with an internal pre-column. Eluents were mixtures of water acidified with 0.1% of orthophosphoric acid or 0.3% formic acid and methanol. HPLC analyses were made in triplicate. UV spectra were recorded on a Cary3 (Varian) spectrophotometer. A 1-cm path quartz cell was used in all the experiments. The reference beam blank was Milli-Q water in all cases.

3. Results and discussion

3.1. Chemical and spectral characteristics of PHFs

The PHFs chosen in this study had different origins. PG, PHF1 and PHF2 were obtained by the hydrolysis of animal protein, while PHF3 was from vegetal protein. All products were commercially available. The hydrolytic process used in the PHF isolation is unknown, but it is generally a chemical–enzymatic mixed process. The chemical analyses of the PHFs are reported in Table 1, and the UV–visible spectra recorded at 800 mg total organic carbon L^{-1} are presented in Fig. 1. The PHFs exhibited similar properties regarding pH, total organic carbon, nitrogen and C/N ratio; however, PG showed some differences. It had the highest percentage of organic matter, about 95% compared to 74.2–88.2% for PHF1–PHF3, the lowest ash (6.2%) and water (3.4%) contents and the highest total nitrogen content (16.4%). It was also enriched with phosphorous and amino acids and poor in phenols. PHF3 had the lowest N content (9.4%) and the highest phenols content, which is in line with the vegetal origin. All PHFs contained minerals (Na, K, Ca, Mg, Fe, Cu, Mn, Zn, Al, Pb, Cd, Cr, Ni and Si) at levels less than 10 mg/kg except for Na, K, Ca and Mg, which may reach 500 mg/kg (concentrated solutions of hydroxides Na, K and Ca were probably used in the hydrolytic process). Fe and Zn were significantly more concentrated in PHF2 than in the other PHFs. The degree of hydrolysis was 32, 17, and 6% for PHF1, PHF2 and PG, respectively. The UV–visible spectra showed quite similar shapes with pronounced maxima at 320 and 270 nm; however, the absorbance intensities were distinct. In particular, PHF3 exhibited about 10 times more absorbance than the other PHFs. PG also absorbed much less than the other PHFs. These differences may be related to the phenol content, as PG was the least absorbing PHF and showed the lowest concentration of phenols; moreover, PHF3 was the most absorbing and contained the highest concentration of phenols.

3.2. Effect of PHFs on the substrate phototransformation

First, all the substrates were irradiated in pH 6.5 buffered Milli-Q water to evaluate the rate of direct photolysis under the chosen experimental conditions. The loss was less than 10% after 10 h of irradiation for fenuron, fluometuron, promethryn and 2,4,6-trimethylphenol and between 10 and 15% after 4–5 h of irradiation for dichlorprop, 2-mercaptobenzothiazole and mesotrione.

The substrates were then irradiated in the presence of the PHFs. The PHFs did not show any noticeable effect on the loss of promethryn, dichlorprop and fluometuron, since the depletion rates did not differ from those measured in buffered Milli-Q water by more than 10%. Due to their high absorption, PHFs are expected to inhibit the direct photolysis of dichlorprop by screen effect. Thus, PHFs had probably a small sensitizing effect on this compound balancing the inhibiting one. In the case of promethryn and fluometuron which did not undergo direct photolysis, the PHFs did not have sensitizing effect. In contrast, the PHFs significantly increased the depletion rates of fenuron, 2-mercaptobenzothiazole, furfuryl alcohol and 2,4,6-trimethylphenol. The rate coefficients are shown in Fig. 2. The case of mesotrione is special and will

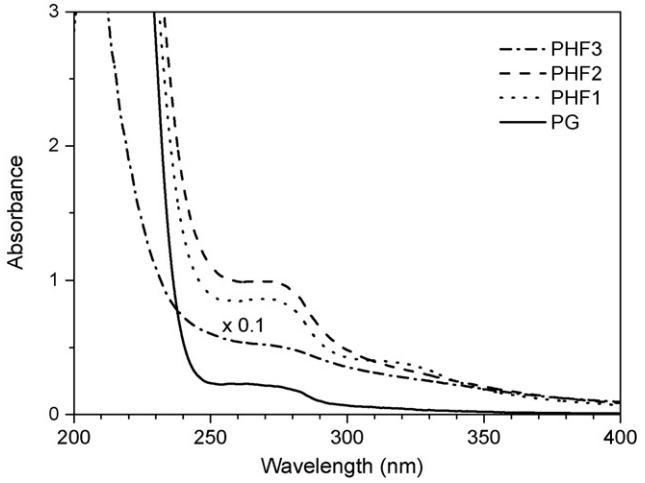

Fig. 1. UV–visible absorption spectra of PHFs in Milli-Q water. (–): PG; (. . .): PHF1; (– – –): PHF2; (–.–.–): PHF3. The PHFs were at a concentration of 800 mg TOC L^{-1}, except PHF3 which was at a concentration of 80 mg total organic carbon L^{-1}.

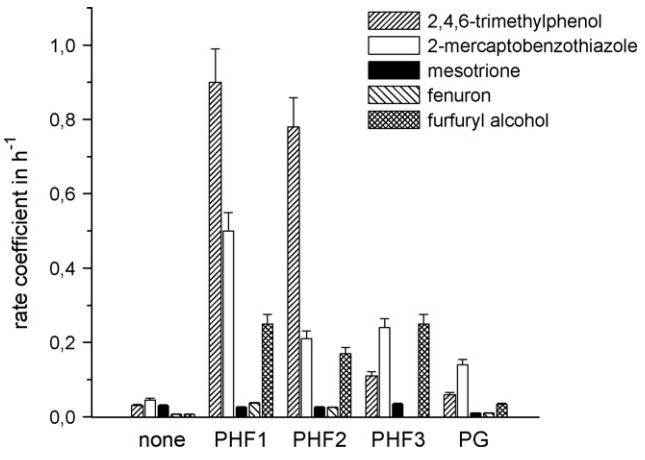

Fig. 2. Rate coefficients in h^{-1} measured for 2,4,6-trimethylphenol, 2-mercaptobenzothiazole, mesotrione, fenuron, and furfuryl alcohol irradiated at a concentration of 9×10^{-5} M in polychromatic light alone or in the presence of PHF1, PHF2, PHF3 or PG (800 mg TOC L^{-1}).

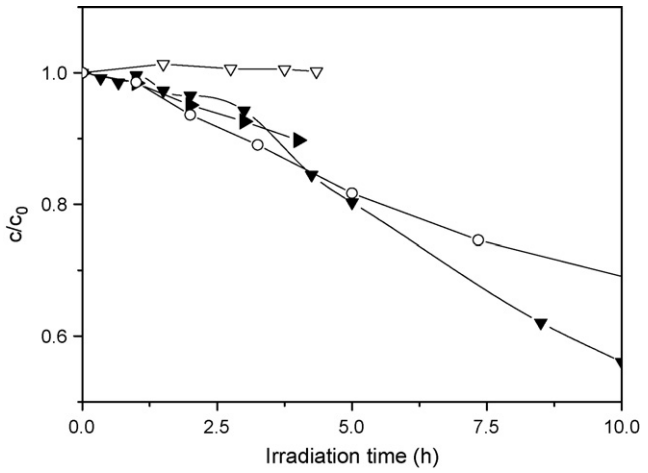

Fig. 3. Consumption curves of mesotrione (9×10^{-5} M) when irradiated in polychromatic light in the presence of PG (▽), PHF1 (▼), PHF2 (►) or PHF3 (○) (800 mg TOC L^{-1}).

be discussed later. For 2,4,6-trimethylphenol, the highest k values (0.7–0.9 h^{-1}) were obtained with PHF1 and PHF2 as sensitizers, with PHF3 and PG giving k values 5–10-fold lower. In the case of 2-mercaptobenzothiazole, the rate coefficient was also higher for PHF1 (0.5 h^{-1}) than for the others PHFs (0.15–0.25 h^{-1}); however, the difference between PHFs was not as important as in the case of 2,4,6-trimethylphenol. Fenuron disappeared slower than the other substrates in the presence of the PHFs, but the accelerating effect was significant. Again, PHF1 was more photosensitizing than the other PHFs. Similar to 2,4,6-trimethylphenol, furfuryl alcohol showed a phototransfomation much faster with PHF1 and PHF2 than with PHF3 and PG. In the case of mesotrione, the effect of the PHFs was more complex. As shown in Fig. 3, PHF1, PHF2 and PHF3 showed an inhibitory effect in the early stages of the reaction. However, at higher irradiation times (after ≈3 h), the mesotrione loss curve showed an auto-accelerated shape. In contrast, PG completely inhibited the photolysis of mesotrione.

3.3. Characterisation of reactive species involved in the photoreactions

To gain better insight into the reaction mechanisms and characterise the reactive species involved, we used the scavenging technique. 2-Propanol was used to trap the hydroxyl radical ($k = 1.9 \times 10^{10}$ M^{-1} s^{-1}) [15], and the azide anion (N_3^-) was used to scavenge singlet oxygen ($k = 7.9 \times 10^8$ M^{-1} s^{-1}) [8]. This chemical also traps hydroxyl radicals (1.2×10^{10} M^{-1} s^{-1}) [15] and triplet excited states [16]. The inhibitory effects of these scavengers are reported in Table 2. Moreover, the rate constants of reaction of the substrates with singlet oxygen were measured or obtained from the literature. The values collected are shown in Table 3.

First, the addition of 2-propanol did not affect the rate of substrate transformation. It can be concluded that hydroxyl radicals are not involved in the PHF-mediated phototransformation. Either these species are negligibly formed or they are efficiently trapped by the PHFs due to their high concentration in the reaction mixtures. PHFs were previously shown to generate singlet oxygen upon excitation [7]. Thus, we first focused on these species, and we used the results obtained with furfuryl alcohol to estimate the rate of singlet oxygen photoproduction in our conditions. Since the loss of furfuryl alcohol is not affected by 2-propanol (5×10^{-3} M), it can be concluded that the observed consumptions are exclusively due to singlet oxygen.

Table 2
Influence of additives on the rates of mesotrione, 2-mercaptobenzothiazole and 2,4,6-trimethylphenol phototransformation in the presence of PHFs. $(r_S)_{additive}$ is the rate of S loss in the presence of additive, and r_S is the rate of S loss in the absence of additive. Ratios $(r_S)_{additive}/r_S$ are given ±20%.

Substrate	Conditions	$(r_S)_{additive}/r_S$
Mesotrione	PHF1	1
	PHF1 + 2-propanol (0.085 M)	1
	PHF1 + sodium azide (9×10^{-3} M)	0.4
2-Mercaptobenzothiazole	PG	1
	PG + 2-propanol (0.085 M)	1
	PG + azide (9×10^{-3} M)	1
	PHF1	1
	PHF1 + 2-propanol (0.085 M)	1
	PHF1 + sodium azide (9×10^{-3} M)	0.4
2,4,6-Trimethylphenol	PG	1
	PG + azide (3×10^{-3} M)	0.5
	PHF1	1
	PHF1 + 2-propanol (0.085 M)	1
	PHF1 + azide (3×10^{-3} M)	0.5
	PHF1 + FFA (3×10^{-3} M)	1
	PHF2	1
	PHF2 + azide (3×10^{-3} M)	0.5
	PHF3	1
	PHF3 + azide (3×10^{-3} M)	0.5

The rate of furfuryl alcohol loss can be written as

$$r_{FFA} = r_{SO} \frac{k_{FFA}^{OS}[FFA]}{k_d + k_{FFA}^{OS}[FFA]} \quad (3)$$

and the rate coefficient can be written:

$$(k_{PHF})_{FFA} = r_{SO} \frac{k_{FFA}^{OS}}{k_d + k_{FFA}^{OS}[FFA]} \quad (4)$$

For FFA equal to 10^{-4} M, $k_{FFA}^{OS}[FFA] << k_d$. Using relationship (4) and the k_{FFA} values given in Fig. 2, r_{SO} can be approximated to $(5 \pm 1) \times 10^{-4}$, $(4 \pm 1) \times 10^{-4}$, $(5 \pm 1) \times 10^{-4}$ and $(7 \pm 2) \times 10^{-6}$ M h^{-1} for PHF1, PHF2, PHF3 and PG, respectively.

2,4,6-Trimethylphenol reacts with singlet oxygen ($k_{TMP} = 6.3 \times 10^7$ M^{-1} s^{-1}) [17], oxidant triplet excited states [10,18] and radicals. Since the rate of 2,4,6-trimethylphenol is 3–5-fold higher than that of furfuryl alcohol, even though the k_{TMP} is two times lower than the k_{FFA}, one can conclude that the contribution of singlet oxygen in the degradation of 2,4,6-trimethylphenol is minor at best. To confirm this, we investigated the effect of furfuryl alcohol (3×10^{-3} M) on the PHF-mediated phototransformation of 2,4,6-trimethylphenol. Furfuryl alcohol did not affect the rate of 2,4,6-trimethylphenol loss, even though it is expected to trap about 50% of the singlet oxygen at the concentration used. This result provides evidence that 2,4,6-trimethylphenol reacts with a species other than singlet oxygen or hydroxyl radicals. The addition of azide (3×10^{-3} M) decreased the rate of 2,4,6-trimethylphenol by 50% for the four PHFs. This inhibitory effect can be attributed to the scavenging of triplet excited states by azide and shows that 2,4,6-trimethylphenol

Table 3
Rose Bengal photosensitized oxidation of substrates (9×10^{-5} M). The bimolecular rate constants, k_S^{OS}, were evaluated using furfuryl alcohol as a reference.

Substrate	Disappearance in % (irradiation time)	k_S^{OS} (M^{-1} s^{-1})
Furfuryl alcohol	(19 ± 2)% (5 min)	1.2×10^8 [8]
2-Mercaptobenzothiazole	(9 ± 2)% (10 min)	$(3.9 \pm 0.8) \times 10^7$
Prometryn	(12 ± 2)% (7 h)	$(8.4 \pm 1.4) \times 10^5$
Mesotrione	(8 ± 2)% (7 h 50 min)	$(5.3 \pm 1.3) \times 10^5$
Fluometuron	<5% (7 h)	$<10^5$
Dichlorprop	<5% (7 h)	$<10^5$
2,4,6-Trimethylphenol		6.3×10^7 [17]

is mainly oxidized by these species. From this hypothesis, the rate of 2,4,6-trimethylphenol phototransformation can be written as

$$r_{TMP} = r_T \frac{k'_{TMP}[TMP]}{k_{O_2}[O_2] + k'_{TMP}[TMP]} \quad (5)$$

and the rate coefficient as

$$(k_{PHF})_{TMP} = r_T \frac{k'_{TMP}}{k_{O_2}[O_2] + k'_{TMP}[TMP]} \quad (6)$$

where r_T is the rate of triplet excited state formation, kO_2 is the rate constant of triplet deactivation by oxygen ($\sim 10^9\ M^{-1}\ s^{-1}$), and k'_{TMP} is the bimolecular rate constant of reaction between the triplet and 2,4,6-trimethylphenol. Taking $kO_2 \sim k'_{TMP}$ [10], r_T values are computed to be $(3 \pm 1.0) \times 10^{-4}\ M\ h^{-1}$ for PHF1 and PHF2, $(4.0 \pm 1.0) \times 10^{-5}\ M\ h^{-1}$ for PHF3 and $(2.0 \pm 0.5) \times 10^{-5}\ M\ h^{-1}$ for PG. These values are consistent with those obtained for r_{SO} (see above).

2-Mercaptobenzothiazole showed rate coefficients higher or close to those of furfuryl alcohol for PHF1, PHF2 and PHF3 (see Fig. 2), whereas its bimolecular rate constant of reaction with singlet oxygen is 3-fold less (see Table 3). Again, one can conclude that the contribution of singlet oxygen to 2-mercaptobenzothiazole phototransformation is at best one-third of the overall contribution in the case of PHF2 and PHF3 and one-sixth in the case of PHF1. After subtraction of the part of singlet oxygen to the overall rate coefficient, the result is $0.42\ h^{-1}$ for PHF1 and $0.12\ h^{-1}$ for PHF2 and PHF3. This remaining contribution is due to triplet excited states or possible radicals. In the hypothesis that only triplet excited states are involved in the reaction, one estimates that the bimolecular rate constant of reaction of 2-mercaptobenzothiazole with triplet is $\sim 5 \times 10^8\ M^{-1}\ s^{-1}$.

The rate coefficients measured for fenuron were the smallest. This compound does not react measurably with singlet oxygen, thus only the contributions of triplet excited states or radicals must be considered. Comparing the rate coefficient of fenuron with that of 2,4,6-trimethylphenol gives a bimolecular rate constant of reaction of fenuron with triplet $\sim 6 \times 10^7\ M^{-1}\ s^{-1}$.

In the case of mesotrione, the PHFs showed an inhibitory effect at the early stages of the reaction. This influence is explained by the facts that the phototransformation of mesotrione involves the triplet excited state, which can be reduced by H-donor molecules [19], and that PHFs contain a variety of H-donor groups. The accelerating effect observed after several hours of irradiation in the case of PHF1, PHF2 and PHF3 might be due to the accumulation of photosensitizing photoproducts of mesotrione. This point needs further investigation.

3.4. Comparison of PHFs activity

We previously showed that PHF1 and PHF2 obtained from animal protein are able to photosensitize the production of singlet oxygen; we show here that PHF3 from vegetal origin is also able to photosensitize the production of singlet oxygen. Only PG, which is also produced from animal protein, failed. Singlet oxygen is not the only photo-oxidant generated by the PHFs. Indeed, in the case of PHF1 and PHF2, triplet excited states seem capable of oxidizing two of the probe molecules, 2,4,6-trimethylphenol and 2-mercaptobenzothiazole, and one of the pesticides, fenuron. As the rate of singlet oxygen is close to the rate of triplet excited state production, one may suggest that triplet excited states generating singlet oxygen are the oxidants. With PHF3, the rate of oxidant triplet excited states production is ten times lower than the rate of singlet oxygen production. Thus, only a small amount of the triplets are oxidants.

PHFs are extracted from proteins; they are complex mixtures, the composition of which remains unknown. One of their characteristics is the high proportion of N (~one N atom for three C atoms), due to their protein origin. The photosensitizing properties may have different origins; the photosensitization might be due to the presence of organic compounds, such as unsaturated molecules bearing heteroatoms N and/or O, showing electrophylic reactivity in the excited state and for which the triplet lifetime is long enough to allow reaction with substrates and energy transfer to oxygen. These compounds might be extracted directly or produced during the isolation process via condensation reactions between nitrogenated compounds and carbonyl, for instance. The reaction between amino acids and carbohydrates is well known. It yields melanoidins, which are brown mixtures that exhibit photosensitizing properties [20]. Inorganic species in association with organic chemicals are potential photosensitizers too.

The differences in reactivity of the PHFs are not easy to rationalize on the basis of the data and analyses presented here. It is however obvious that PG, which is by far the least absorbing PHF and shows the lesser degree of hydrolysis, is the least efficient. The origin of the PHF is not a crucial parameter because PHF3, which is of vegetal origin, gives similar reactions as PHF1 and PHF2, which are obtained from animal sources. Moreover, PG, which is from animal sources like PHF1 and PHF2, yields different reactions. The point could be the method by which the PHFs are produced, i.e., aqueous extraction, chemical hydrolysis—acid or basic, enzymatic hydrolysis, or chemical followed by an enzymatic hydrolysis. In this scheme, hydrolytic processes would play an important role by liberating chromophores capable of producing singlet oxygen upon light absorption.

In conclusion, we showed that in water solutions, PHFs may have an influence on the phototransformation of organic compounds both through the production of singlet oxygen and through that of oxidant triplet excited states. These photosensitizing properties likely depend more on their production process than on their origin. The PHFs may also affect the photolysis of organic compounds through their H-donor properties. Their overall influence is thus complex and should be investigated systematically before use. Studies will be undertaken on leaf surfaces to understand what could happen after foliar application of an herbicide mixed with PHFs.

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