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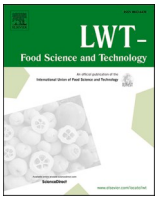

Photo-degradation of alfalfa saponins by UV–visible multi-wavelength irradiation

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ABSTRACT

A photochemical treatment with UV multi-wavelength irradiation was applied to investigate the degradation of the saponins mixture obtained from alfalfa leaves. The photo-degradation was studied in an aqueous solution at a pH range of 4–7 and temperature range of 20–80 °C. The samples were irradiated for 80 min using a mid-pressure mercury lamp with emission wavelengths between 250 and 740 nm. The dependence of the absorbed radiation on the concentration of alfalfa saponins was evaluated, and a linear dependence was found for the range of concentrations experimented throughout the photo-degradation. The alfalfa saponins mixture absorbed radiation between 470 and 610 nm with a maximum absorption peak at 543 nm. This means that any lamp emitting within this wavelength range could produce some degree of photo-degradation. A reaction mechanism was proposed, matching the experimental pseudo-first-order kinetic model that best fitted the evolution of the saponins mixture concentration. The photo-degradation rate was found to increase with increasing temperature and decreasing pH value, causing a reduction of 80% in the concentration of the alfalfa saponins mixture after 80 min at pH 4 and 80 °C.

1. Introduction

Alfalfa, called *Medicago sativa* L. in binomial nomenclature, is a productive and adaptable forage crop. It is widely grown throughout the world as pasture and hay, and is mainly fed to livestock. Owing to their high protein content, alfalfa leaves have promising potential for human consumption (Huang et al., 2018; Hadidi et al., 2020). Unfortunately, they also contain saponins, which are mixtures of many different compounds, such as medicagenic acid, zanhic acid, and soyasaponin (Nowacka & Oleszek., 1992), these being secondary metabolites that act as a chemical barrier or shield in the plant defense system to counter pathogens and herbivores (Cheok et al., 2014). Saponins are mainly mixtures of many derivatives of terpene, most of them being glycosides of one of the many possible triterpenes with different substituents such as hydroxyl, hydroxymethyl, carboxyl and acyl groups. Thus, since the number of different compounds that can be present in the saponins mixture is almost infinite, it does not make much sense to try to identify and quantify every compound. Knowing the source of the mixture and the way the saponins mixture was produced is worthier and more useful in order to characterize the specific mixture.

The use of alfalfa leaves in food is limited because of their anti-nutritional and undesirable taste properties, some of which may be due to the high levels of saponins, which taste bitter (Oleszek, 2002; Kalac et al., 1996), and limits the accessibility of alfalfa for both humans and animals (Savage, 2016; Wang & Kinsella, 1976). Although saponins have some health benefits (Singh et al., 2017), they are also known as anti-nutrients at high levels due to having hemolytic and inhibitory activities. Additionally, they can affect muscosal cells permeability in the small intestine, and thus, reduce the bioavailability of nutrients and decrease enzyme activity (Savage, 2016).

If there were a way to remove saponins from alfalfa leaves, they could become a promising as a food for mankind. Proteins and other nutrients in alfalfa can be extracted as aqueous solutions, but unfortunately saponins are also extracted (Oleszek, 1988). Some researchers have employed different processes to degrade these saponins, including steaming (Hadidi et al., 2019), microwaving and cooking (Heng et al., 2006; Ruiz et al., 1996; Shi et al., 2009; Tarade et al., 2006). The extraction processing conditions, including heat, pH, solvent and food matrix, can affect the saponins content of the extracted obtained, as well as its stability (Güçlü-Üstündağ & Mazza, 2007).

Ultraviolet irradiation has been widely applied to disinfect drinking water, air and food surfaces (Falguera et al., 2011). Such undesired

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substances as patulin (Ibarz et al., 2014, 2017; Garvín et al., 2015), ochratoxin (Ibarz et al., 2015; Ibarz et al., 2015), thiabendazole (Ibarz, Garvín, Rojas, et al., 2016) and 5-hydroxymethylfurfural (Aguilar et al., 2015, 2016a) in food liquids are degraded by irradiation with ultraviolet radiation. Thus, it seems interesting to check whether this technique could degrade the saponins in alfalfa extracts. If so, this would allow the rest of the nutrients in alfalfa, previously extracted to an aqueous solution, to be used as food.

The lamps used most frequently for ultraviolet irradiation are low- and medium-pressure mercury vapor lamps. The low-pressure lamps emit almost exclusively at the germicidal wavelength of 254 nm, whereas medium-pressure lamps emit polychromatic light with emission bands in both the ultraviolet and visible light regions (Falguera et al., 2011). Medium-pressure lamps can provoke the same germicidal effect at 254 nm and do so even faster due to the fact that their total emitting power is usually much higher than the low-pressure ones, while other additional chemical changes can also be caused by some of the other wavelengths absorbed by the irradiation target (Garvín et al., 2015).

Some of the photo-degradations published in the literature propose one specific reaction mechanism that matches the kinetical model experimentally obtained (Garvín et al., 2015; Ibarz et al., 2014, 2015, 2016a, 2016b). Although authors were never able to conclude whether the reaction mechanism that matched the experimental data were true or not, in these kind of studies the proposed mechanism only tries to help to understand how the reaction seems to take place, mainly ruling out other possible mechanisms that do not match the experimental data.

So, the main objective of this study was to evaluate whether the saponins, unfortunately present in alfalfa extracts, could be degraded by UV–Visible light in order to know whether the saponins could be removed from industrial alfalfa protein previously to be destined as human food. Secondary objectives were to determine the influence of pH and temperature in the reaction rate, to find the kinetic model that fits the experimental data and to propose a reaction mechanism that matches the kinetic model obtained.

2. Materials and methods

2.1. Materials

Fresh alfalfa (*Medicago sativa* ssp. *Falcate ver. Martyn*) leaves were harvested from a standard crop (sandy loam soil, pH 8.4 and 3.5% organic matter) in Lleida, Spain (N 41° 37′, E 0° 35′ at an altitude of 177 m) in June 2019. Oleanolic acid and vanillin were supplied by the Sigma Chemicals Co. (Missouri, USA). All other reagents and chemicals were of analytical grade from Merck Chemical Co. (Darmstadt, Germany) and Panreac Química S.A. (Barcelona, Spain).

2.2. Extraction of saponins from alfalfa leaves

These specific alfalfa leaves were used to obtain their saponins mixture following the extraction method first proposed by Oleszek (1988) but improved by Hadidi et al. (2020) by using sonication during the extraction. This consists of defatting dried powdered alfalfa leaves with hexane in a Soxhlet extractor for 2 h and then applying the extraction method with sonication as described by Hadidi et al. (2020). Thus, the extraction took place by adding an aqueous solution of 78.2% ethanol (v/v) at a solvent/raw material ratio of 11.4 mL/g and applying an ultrasonic power of 112.0 W with an ultrasonic cleaner bath (10 L, Skymen Co., Guangdong, China) for 2.84 h at 76.8 °C. After extraction, the remaining alfalfa leaf powder was removed by filtration and the remaining solvent in the solution was separated in a rotary evaporator at 50 °C (SCI100–S, Scilogex, Hartford, USA). It was then diluted with methanol up to a concentration of 35% (w/v). For purification, the solution was loaded into a pre-conditioned C18 column with 35% (w/v) methanol (6 cm × 10 cm, 55 p.m., 50 g) (Waters Associates, Frankfurt, Germany). The column containing the saponins mixture was first washed using 200 mL of 35% (w/v) methanol to eliminate sugars and phenolic compounds. Then the mixture was eluted by using a second volume of 200 mL of 35% (w/v) methanol. The methanol was then eliminated in a rotary evaporator and water in a freeze dryer (RevoPro, Millrock Technology, Inc, New York, USA), thus obtaining the solid saponins mixture as a yellowish powder.

2.3. Preparation of saponins solution

The saponins solution to be irradiated was obtained by dissolving the extracted saponins powder in an aqueous methanol solution (20% v/v) up to a concentration of 100 mg/L at a pH value of 4, 5, 6 or 7. Each pH value was set using phosphate/citric acid buffers (McIlvaine). The range of pH studied was set taking into account the expected range for the extracts of green leaves (Arabshahi et al., 2007).

2.4. UV irradiation of samples

The installation used in this work is the same as described in other works (Ibarz et al., 2016; Aguilar et al., 2016). To carry out the irradiation of the samples, 800 mL of the methanol-water solution (20% v/v) was placed in a parallelepiped-shaped methacrylate reactor that measured 12.5 × 10.5 × 10 cm, so that the surface of the sample was 22.5 cm from the lamp and the solution height was 6.4 cm. The irradiation of each solution was carried out in a black chamber containing the reactor and the lamp. The reactor was mixed using a magnetic stirrer. The working temperatures (20, 40, 60 and 80 ± 1 °C) were maintained in the reactor by using a refrigerant coil. A mid-pressure Philips HPM 12 mercury lamp (Philips, Amsterdam, The Netherlands) with a 460 W

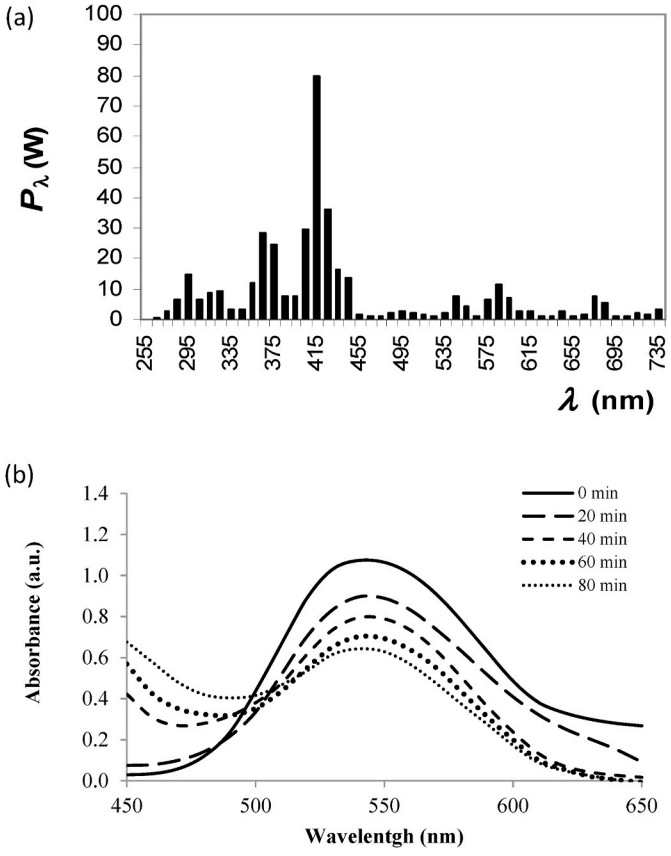

Fig. 1. (a) Emission spectrum for HPM 12 mercury lamp, (b) Evolution of the absorption spectrum with irradiation time for a alfalfa saponin concentration of 100 mg/L at pH = 4 and 20 °C.

nominal power was employed, emitting in a wavelength range of 250–740 nm (Fig. 1). In order to ensure a constant UV emission, the lamp was turned on 10 min before placing the samples in the reactor to be irradiated. Every solution was irradiated for 80 min and a 2 mL sample was taken out every 10 min in order to analyze its total saponin content and, in some cases, to obtain the absorption spectrum.

2.5. Determination of the saponin content

One mL of each sample was mixed with 1 mL of vanillin solution in ethanol (0.8% w/v) and 10 mL of sulphuric acid in water (72% v/v). A control sample using ethanol was also prepared. Then the samples were heated in an orbital shaker at 60 °C for 10 min. Vials of mixtures were instantly put in an ice bath for 5 min to stop further reaction, and the absorbance was measured using a spectrophotometer (6850 UV/Vis, Jenway Co., Jenway, United Kingdom) at 520 nm by the control sample containing methanol as zero absorbance. The absorbance value was converted into saponins content by using the standard curve, ranging from 10 to 1500 μg/mL, previously obtained with oleanolic acid. The results were expressed as mg of total saponins per L of solvent (Navarro del Hierro et al., 2018).

2.6. Absorption spectra

The absorption spectra were obtained for the solution of 100-mg/L alfalfa saponins at pH = 4 and 20 °C before being irradiated and 20, 40, 60 and 80 min post-irradiation. A spectrophotometer (6850 UV/Vis, Jenway Co., Jenway, United Kingdom) and a 1-cm wide quartz cell were used to scan all the wavelengths from 250 to 750 nm (Ibarz et al., 2014).

2.7. Actinometric analysis

Actinometry was used to determine the actual power of the lamp using the photo-chemical decomposition of oxalic acid in the presence of uranyl cation (Ibarz et al., 2014).

2.8. Photo-degradation of the alfalfa saponins kinetics model

The same three-stage degradation mechanism described by Ibarz et al. (2014 and 2015) is proposed. This reaction mechanism has been widely accepted and fits the usual kinetics (zero, first and pseudo-first order) experimentally obtained for photo-degradations (Garvín et al., 2015). The first stage consists of the absorption of photons by the molecules in the saponins mixture turning into excited versions of the same molecules (saponins*). Then the excited molecules can either decline to their fundamental state or degrade to form photo-products. This three-stage mechanism can be represented by the following reactions:

$$Saponins \xrightarrow{P_{abs}} Saponins^{*}$$

$$Saponins^{*} \xrightarrow{k_D} Saponins$$

$$Saponins^{*} \xrightarrow{k} Photo-products$$

where, P_{abs} is the total radiation absorbed per volume unit by the irradiated solution, which in the case of an aqueous solution of only saponins, coincides with the radiation absorbed by the molecules that form the saponins mixture. The units of P_{abs} have to be Einsteins (mols of photons) per unit of volume and time. k_D is the kinetic constant of the declination stage and k is the kinetic constant of the degradation of the excited molecules to photo-products.

In order to obtain the mathematical relation for the reaction rate, a stirred batch reactor was considered. The relation between the concentration of excited saponins (C_{S^*}) can be obtained considering a pseudo-steady state for these substances:

$$C_{s^*} = \frac{1}{k_D + k} P_{abs} \tag{1}$$

The reaction rate in a batch reactor is the variation of the concentration of saponins with time. For this reaction mechanism, it can be expressed as:

$$r_s = \frac{dCs}{dt} = -P_{abs} + k_D C_{s^*} = -\frac{k}{k_D + k} P_{abs} = -K_s P_{abs} \tag{2}$$

From Equation (2), K_S is defined as:

$$K_S = \frac{k}{k + k_D} \tag{3}$$

K_S is the quantum yield of the absorbed radiation. It means that for each photon of light (Einstein) absorbed by the molecules of alfalfa saponins (P_{abs}), a fraction K_S of this energy is used for the photo-degradation path, while the rest (1-K_S) is wasted and does not cause any chemical change in the molecules that absorb that photon of light. The ratio k_D/k can be obtained from Equation (3). This ratio shows how fast the declination stage of the reaction mechanism (second stage) is compared to the photo-degradation stage (third stage). Obviously, the higher the value of this ratio (k_D/k), the lower is the efficiency of the absorbed energy in order to achieve the photo-degradation.

According to Garvín et al., 2015, the incident radiation on any surface at any depth (z) from the reactor surface ($P(z)$) and the absorbed radiation per volume unit (P_{abs}) can be evaluated from the following equations:

$$P(z) = \sum_{\lambda} \frac{W_\lambda / L}{4\pi} \int_{x=0}^{x=A} \int_{y=0}^{y=B} \int_{y_L=y_0}^{y_L=y_0+L} \frac{e^{-\mu_\lambda \frac{z}{\sin\beta}}}{D^2} dy_L dy dx \tag{4}$$

$$P_{abs} = \frac{1}{V} \sum_{\lambda} \int_{x=0}^{x=A} \int_{y=0}^{y=B} \int_{z=0}^{z=C} \int_{y_L=y_0}^{y_L=y_0+L} \frac{W_\lambda / L}{4\pi D^2} \exp\left(-\mu_\lambda \frac{z}{\sin\beta}\right) \left(1 - \exp\left(\mu_\lambda \frac{dz}{\sin\beta}\right)\right) dy_L dx dy dz \tag{5}$$

where, W_λ is the emitted power of the whole lamp for the specific wavelength λ, L is the length of the lamp, x and y are the axes of the surface of the reactor, y is the axis parallel to the lamp; A, B and C are the lengths of the reactor according to the x, y and z axes respectively, y_L is the distance between any specific point in the lamp and the beginning of this lamp located at y_0 according to the y-axis, β is the angle defined between each specific point in the reactor and the specific point considered in the lamp, D the distance between both specific points, and V is the volume of the reactor.

Equation (4) allows the incident radiation at the surface of the reactor to be calculated for z = 0 ($P(0)$) along with the incident radiation at the bottom of the reactor for $z = C$ ($P(C)$), where C is the total depth of the reactor.

In order to facilitate the solution to Equation (5), it is useful to divide the reactor into a number of n layers (Garvín et al., 2015):

$$P_{abs}=\frac{1}{V}\sum_{\lambda}\sum_{i(z)=1}^{i(z)=n}\int_{x=0}^{x=A}\int_{y=0}^{y=B}\int_{y_L=y_0}^{y_L=y_0+L}\frac{P_{emit,\lambda}/L}{4\pi D^2}dy_L\left[e^{-\mu_\lambda D_{i-1}}-e^{-\mu_\lambda(D-D_0)}\right]dxdy \quad (6)$$

Equations (4)–(6) need to be calculated for each saponins concentration value (or absorption coefficient, μ_λ) using a numerical method.

Since the irradiation process is carried out in a stirred batch reactor of volume *V,* Equation (2) should be applicable if this reaction mechanism is correct.

In order to solve this equation, the relation between P_{abs} and the saponins concentration needs to be known. Taking into consideration the mathematical relation between P_{abs} and the concentration of the absorbing substance predicted by Garvín et al., 2015, if the working concentration range is neither too high nor too low, the expected dependence should be a straight line with a non-zero origin ordinate:

$$P_{abs}=a+bC_s \quad (7)$$

Combining Equations (2) and (7), Equation (8) is obtained:

$$\frac{dCs}{dt}=-K_s(a+bC_S) \quad (8)$$

By integrating from the initial concentration of saponins (C_S^0) to their concentration for a specific time t (C_S), we obtain:

$$ln\left(\frac{a+bC_S}{a+bC_S^0}\right)=-bK_St \quad (9)$$

Accordingly, the evolution of C_S with irradiation time can be expressed as below:

$$C_S=-\frac{a}{b}+\left(\frac{a}{b}+C_s^0\right)\exp(-bK_St) \quad (10)$$

where $b{\cdot}K_S$ is the kinetic constant (m_S) for the pseudo-first order photodegradation of alfalfa saponins:

$$m_S=bK_S \quad (11)$$

2.9. Statistical analysis

The experimental data were fitted to several kinetic and mathematical models with a significance level of 0.05 using the Statgraphics (ver 5.1, STSC Inc. USA). Both the UV radiation treatments and the sample analysis were performed in duplicate.

3. Results and discussion

3.1. Actinometry

The actinometric analysis showed that a power of $1.38{\cdot}10^{-3}$ E/s was emitted by the HPM 12 lamp.

3.2. Absorption spectrum

Fig. 1a illustrates the emission spectra of the lamp used and Fig. 1b indicates the evolution of the absorption spectra of a solution with an initial alfalfa saponins concentration of 100 mg/L. All these absorption spectra were obtained at pH 4 and 20 °C, and all showed absorption wavelengths in the 470–610 nm range with a maximum absorption peak at 543 nm, in agreement with the findings of Hu et al. (2012). The absorption spectra for the initial alfalfa concentration of 100 mg/L were also obtained at pH 5, 6 and 7. However, they showed roughly the same tendency as the absorption spectrum at pH 4 shown in Fig. 2. This means that the UV absorption of the 100 mg/L solution of alfalfa saponins does not depend on the pH value in the range 4–7. Comparing the emission and absorption spectra, it can be seen that within a range of 470–610 nm, the radiation emitted by the lamp can be absorbed by alfalfa saponins. Table 1 shows the extinction coefficients for each wavelength

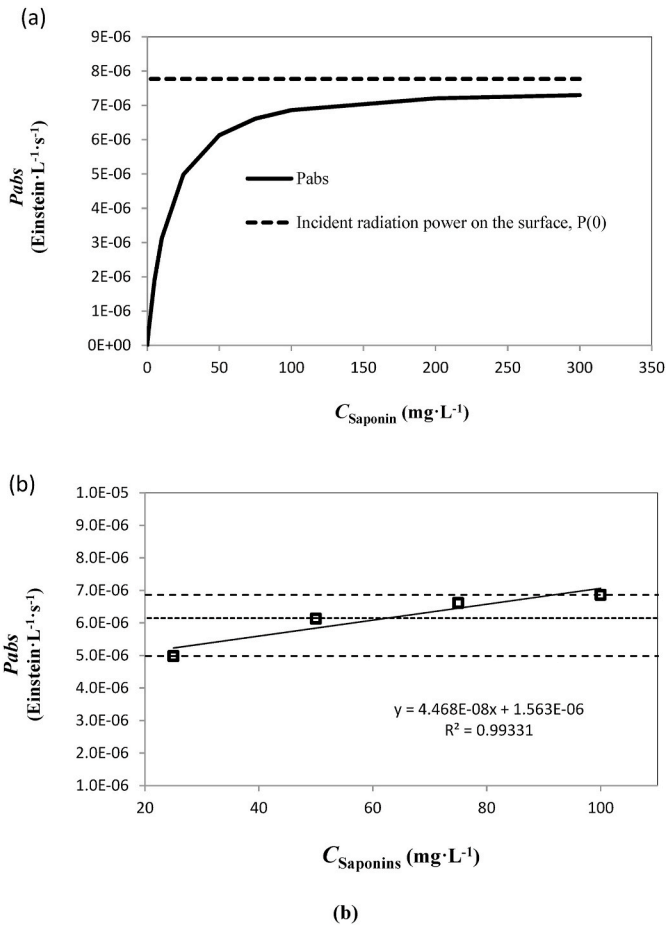

Fig. 2. Absorbed radiation as a function of alfalfa saponins concentration: (a) from 0 to 300 mg L^{-1}, and (b) from 25 to 100 mg L^{-1}, showing the parameters a and b fitted by linear regression ($P_{abs}=a+bC_S$).

Table 1
Energy emitted by the used lamp (*P*emit), mass extinction coefficients (ε_λ) of alfalfa saponins and absorption coefficients (μ_λ) for a 100-mg·L^{-1} solution at different wavelengths (λ).

λ (nm)	P_{emit} (W)	$\varepsilon_\lambda{\cdot}10^3$ (L·mg^{-1} cm^{-1})	μ_λ (C = 100 mg L^{-1}) (cm^{-1})
255	0.1792	11.61	1.161
485	0.8946	2.07	0.207
495	2.0580	4.74	0.474
505	1.7900	9.39	0.939
515	1.4315	14.62	1.462
525	0.9842	19.83	1.983
535	1.7900	23.05	2.305
545	6.4430	24.02	2.402
555	3.4896	23.81	2.381
565	1.0738	22.57	2.257
575	5.5476	20.29	2.029
585	9.3060	17.22	1.722
595	5.9061	13.82	1.382
605	5.4963	10.50	1.050
615	2.1477	8.08	0.808
625	1.0738	6.93	0.693
635	0.9842	6.22	0.622
645	5.3170	5.76	0.576
655	1.0738	5.48	0.548
665	1.3419	5.25	0.525
675	6.4430	4.95	0.495
685	4.5634	4.44	0.444
695	0.8050	3.73	0.373
705	0.8050	2.23	0.223

(ε_λ) obtained from the data corresponding to the initial solution in Fig. 1b by applying the Lambert–Beer equation. It also shows the radiation power emitted by the lamp ($P_{emit,\ \lambda}$) at each wavelength within the range considered.

3.3. Dependence of P_{abs} on the content of alfalfa saponins

Table 1 includes the absorption coefficients (μ_λ) for the initial alfalfa saponins concentration of 100 mg/L at each wavelength within the emission and absorption ranges. The absorption coefficients varied from 0.207 to 2.402 cm^{-1}. It is known that for high values of absorption coefficients, all the radiation reaching the surface of the reactor will be absorbed in the first few millimeters (λ).

Fig. 2a indicates the values calculated for P_{abs} for the specific experimental installation and the extinction coefficients obtained for alfalfa saponins. It can be seen that the relation is almost linear for low concentrations and trends towards an asymptote for high concentrations. The asymptote coincides with the value calculated for the incident radiation on the surface of the reactor ($P(0)$), because for high concentrations, all the radiation reaching the surface of the reactor will be absorbed (Garvín et al., 2015). $P(0)$ was calculated to be $7.77{\cdot}10^{-6}$ E $s^{-1}{\cdot}L^{-1}$. P_{abs} could be considered a constant value for alfalfa saponin concentrations between 100 and 300 mg L^{-1}, which is below but near to $P(0)$. P_{abs} varies from $6.86{\cdot}10^{-6}$ to $7.30{\cdot}10^{-6}$ E L^{-1} s^{-1} for 100 and 300 mg L^{-1}, respectively (average $7.12{\cdot}10^{-6}$ E L^{-1} s^{-1}). Fig. 2b illustrates how, as stated above, for the working range of alfalfa saponin concentrations between 25 and 100 mg L^{-1}, P_{abs} follows a linear trend with an ordinate at the origin, thus fitting Equation (7). The figure also shows the fitted values for a and b from Equation (7) ($1.56{\cdot}10^{-6}$ and $4.47{\cdot}10^{-8}$ E mg^{-1} s^{-1}, respectively with a determination coefficient of 0.9933).

Fig. 3 indicates the profile of the incident radiation reaching the surface of the reactor ($P(0)$) and its bottom ($P(C)$), depending on the coordinates of the x and y axes. As expected, the energy that reaches the surface is greater than the energy that reaches the bottom. An average value of $9.49{\cdot}10^{-3}$ W cm^{-2} was obtained for the whole surface of the reactor $P(0)$, independently of the solution concentration. $P(C)$ does depend on the solution concentration. Thus, for a concentration of 100 mg L^{-1}, this value decreases to $1.33{\cdot}10^{-4}$ W cm^{-2} at the bottom of the reactor, which represents 1.4% of the radiation at the surface. The rest is mainly absorbed by the solution (there is a fraction that exits through the lateral walls of the reactor without having been totally absorbed yet), thereby, making the degradation reaction possible. The total energy absorbed by the 100 mg L^{-1} methanol-water alfalfa saponins solution in the entire reaction volume for the considered range of wavelengths is $8.4{\cdot}10^{-3}$ W cm^{-2}.

3.4. Photo-degradation of alfalfa saponins

The evolution of the alfalfa saponins content when a methanol-water solution of 100 mg L^{-1} is irradiated is illustrated in Fig. 4. In all the cases, alfalfa saponins are degraded during the irradiation of the solutions, matching a pseudo-first-order kinetics. Fig. 4a indicates the effect of temperature on the solutions at pH = 4. While at temperatures of 20 °C, the alfalfa saponins concentration decreases by about 50% after 80 min of irradiation, at 80 °C the degradation increases to about 80%. Therefore, as expected, the higher the temperature, the faster the rate of alfalfa saponins photo-degradation. Fig. 4b illustrates the evolution of

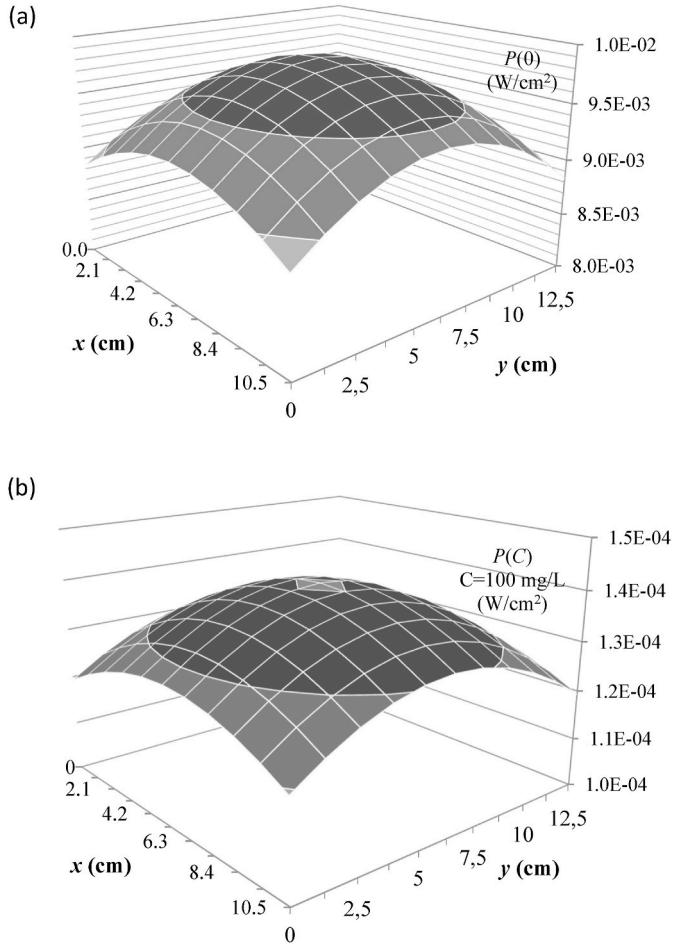

Fig. 3. Incident spectral radiant power: (a) on the surface of the photo-reactor ($P(0)$), and (b) at the bottom of the photo-reactor ($P(C)$) for a 100-mg·L^{-1} alfalfa saponin solution.

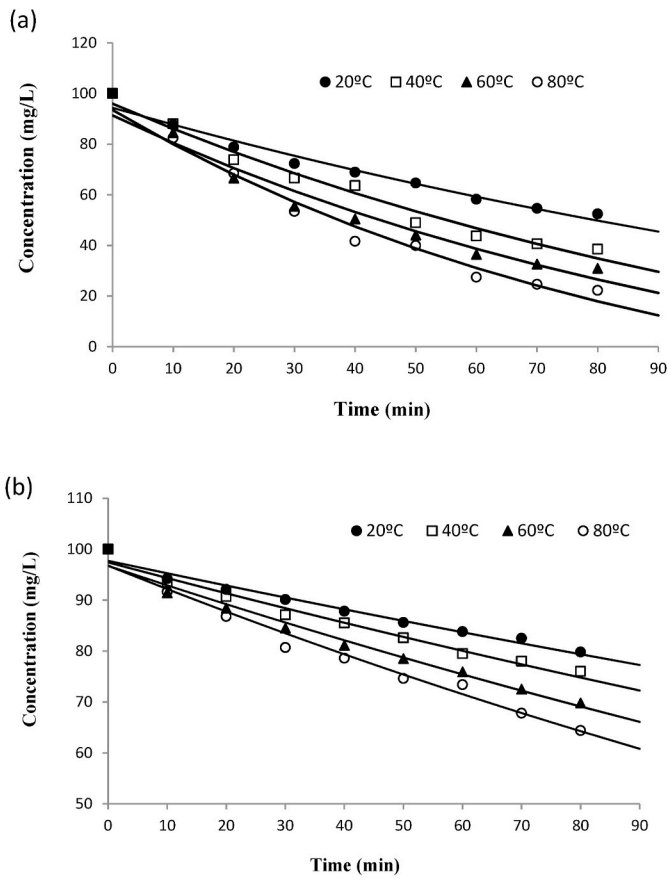

Fig. 4. Evolution of alfalfa saponin concentration with irradiation time at different temperatures: (a) pH = 4 and (b) pH = 7.
Lines show the fitted equation from the pseudo-first-order fits (Table 2).

Table 2
Fitted parameters for the pseudo first-order kinetic photo-degradation of alfalfa saponins in methanol-water solutions at different pH and temperature values.

$$\ln\left(\frac{a+bC_S^0}{a+bC_S}\right) = m_S\, t = b{\cdot}K_S{\cdot}t$$

pH	T (°C)	Intercept	$m_S \cdot 10^4$ (s^{-1})	K_S (mol/Einstein)	k_D/k	R^2
4	20	0.043 ± 0.036	0.88 ± 0.13	0.0039	253	0.9750
	40	0.030 ± 0.055	1.31 ± 0.19	0.0059	170	0.9738
	60	0.066 ± 0.075	1.50 ± 0,26	0.0067	148	0.9638
	80	0.049 ± 0.077	1.85 ± 0.27	0.0083	120	0.9741
5	20	0.032 ± 0.031	0.79 ± 0.11	0.0035	282	0.9770
	40	0.025 ± 0.049	1.11 ± 0.17	0.0050	200	0.9709
	60	0.013 ± 0.029	1.46 ± 0.10	0.0065	152	0.9940
	80	0.025 ± 0.049	1.69 ± 0.17	0.0076	131	0.9871
6	20	0.016 ± 0.017	0.48 ± 0.06	0.0021	464	0.9811
	40	0.022 ± 0.024	0.64 ± 0.09	0.0029	348	0.9776
	60	0.031 ± 0.024	0.71 ± 0.08	0.0032	314	0.9826
	80	0.035 ± 0.035	0.94 ± 0.11	0.0042	237	0.9842
7	20	0.017 ± 0.012	0.31 ± 0.04	0.0014	720	0.9758
	40	0.019 ± 0.015	0.39 ± 0.05	0.0017	572	0.9779
	60	0.025 ± 0.018	0.49 ± 0.06	0.0022	455	0.9808
	80	0.024 ± 0.022	0.59 ± 0.08	0.0026	378	0.9791

solutions at pH = 7 for 20, 40, 60 and 80 °C, showing the same effect of the temperature. After 80 min of irradiation at pH = 7, the alfalfa saponin concentration remained quite high, about 80% at 20 °C and 65% at 80 °C. Thus, comparing the data given in Fig. 4a and b, it can be seen that alfalfa saponins degrade faster at pH = 4 than at pH = 7.

The experimental data of the variation in the concentration of alfalfa saponins with irradiation time, for all the pH and temperatures tested, were fitted to Equation (10). Table 2 shows the fitting parameters and Fig. 4a and b, the fitted lines. Table 2 illustrates how the alfalfa saponins photo-degradation constant (m_S) increases with temperature and decreases with the pH value, which means that they degrade more easily when the acidity of the medium increases for the pH range of 4–7. So having the value of the photo-degradation constant (m_S) and using Equation (3) and the average molecular weight of saponins (500 Da) (Mauro et al., 2021), the value of the quantum yield (K_S) was obtained. Obviously, the quantum yield values (K_S) given in Table 2 show the same trend as the photo-degradation constant (m_S), i.e., they increase with the increase in temperature and with the acidity of the medium. With Equation (3) and the quantum yield values (K_S), it is possible to obtain the relationship between the kinetic constants of the stage of return to the fundamental state and the formation of photoproducts (k_D/k) for the excited molecules of alfalfa saponins as a result of absorbing radiation according to the proposed reaction mechanism. All values of k_D/k ratio calculated in Table 2 are greater than 1, meaning that, in all the cases, the declining step was faster than that of the photo-product formation. It can also be observed that the ratio decreases when the temperature increases, confirming that the efficiency of the photo-degradation increases with temperature. Table 2 also confirms that the higher the temperature, the faster the photo-degradation. Other photo-degradations showed similar facts (Aguilar et al., 2015, 2019; Ibarz et al., 2014, 2015, 2016b). From m_s and K_s values in Table 2, the optimal conditions for degrading alfalfa saponins within the ranges of variables studied are confirmed to be 80 °C and pH = 4.

4. Conclusion

Alfalfa saponins absorb radiation between 470 and 610 nm with a maximum absorption peak at about 543 nm, which means that any lamp emitting in this wavelength range could produce some degree of photo-degradation. Both the temperature and pH of the solutions influenced the extent of the photo-degradation of the alfalfa saponins. The degradation process of the alfalfa saponins was enhanced by increasing the temperature and decreasing the pH value. Within the experimental range for pH and temperature used, the optimal conditions for degrading alfalfa saponins were 80 °C and pH = 4, reaching a decrement of 80% in the primary alfalfa saponin concentration after 80 min. The degradation of alfalfa saponins by multi-wavelength UV irradiation fitted well to a pseudo-first-order kinetic model within the range of concentrations tested. This seems to be caused by the fact that the radiation absorbed by alfalfa saponins in a methanol-water solution follows a straight line with an origin ordinate within the same range of concentration.

CRediT authorship contribution statement

Milad Hadidi: Methodology, Investigation, Visualization, Formal analysis, Writing – original draft, Writing – review & editing. **Alfonso Garvín:** Conceptualization, Methodology, Supervision, Visualization, Formal analysis, Writing – original draft, Writing – review & editing. **Raquel Ibarz:** Conceptualization, Supervision, Visualization, Formal analysis, Writing – original draft, Writing – review & editing. **Albert Ibarz:** Conceptualization, Supervision, Resources, Funding acquisition, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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