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Development of an irradiation equipment to accelerate the degradation of rosé wine in antique green and flint bottles

Jennifer Moriones [a,b,c], Nerea Jiménez-Moreno [b,c], Carmen Ancín-Azpilicueta [b,c], Jonathan Fernández de Ara [a], Beatriz Navarcorena [a], Eluxka Almandoz [a,b,c,**], Irene Esparza [b,c,*]

[a] Asociación de la Industria Navarra, Carretera Pamplona 1, 31191, Cordovilla, Spain
[b] Departamento de Ciencias, Universidad Pública de Navarra, Campus Arrosadía s/n, 31006, Pamplona, Spain
[c] Institute for Advanced Materials and Mathematics (INAMAT^2), Universidad Pública de Navarra (UPNA), Campus de Arrosadia, 31006, Pamplona, Spain

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ABSTRACT

Flint bottles make rosé wines more attractive to the customers and, also, allow them to detect oxidation problems in the color of these wines. Nonetheless, transparent bottles do not protect wines from light. In this work, a device capable of accelerating the degradation of rosé wines using W lamps radiation for short times of exposure has been developed. This equipment has been used to accelerate the color photodegradation of rosé wines, allowing, thus, to identify which parameters can be used as markers of such degradation. The irradiation treatment applied to rosé wines bottled in different types of glass (Flint and Antique Green glass) influenced all the samples. However, the wines treated in Flint bottles displayed more important color variations, especially in color intensity (CI) and hue, than the wines treated in Antique Green bottles. These changes entailed a quality loss of rosé wines that can be appreciated with a naked eye. The yellow component of rosé wines treated in transparent bottles increased the detriment of the red and blue ones. Therefore, color parameters such as CI and a*, together with the total anthocyanin content, seem to be good markers of the loss of quality of rosé wines due to the light effects. The next step will be to find a physical, chemical or physical-chemical protection strategy that, when applied to transparent glass, allow to achieve the light-filtering properties of green glass bottles.

1. Introduction

Flint bottles are not the best option to protect wines from light, but different commercial reasons force the use of these transparent containers, especially for white and rosé wines. Flint bottles make this product more attractive to the customer, but also, in this way, the customer can detect wine oxidation problems through the perception of its color (Ghidossi et al., 2012; Maury et al., 2010). However, there is consensus that amber and green bottles provide good protection against UV–Vis and short wavelengths in the visible region, whereas blue bottles show weak defense and wines in flint containers are strongly affected under these conditions (Dias et al., 2013; Pajean et al., 2006; Perscheid et al., 1978). This is particularly relevant in the case of rosé wines, mainly due to their susceptibility to light, which affects its color and sensory properties (Lan et al., 2021; Benucci, 2020; Stávek et al., 2012).

UV–Visible radiation determines the evolution of different phenolic compounds in wine, like flavonoid pigments (Benucci, 2020) and other pigments such as xanthylium salts (Es-Safi et al., 2001). Nevertheless, the phenolic compounds, which are responsible for the color of rosé wine, are not the only compounds that can be altered by the action of UV–Vis radiation. Contrasted studies have demonstrated that solar radiation, and especially wavelengths in the range 370–450 nm can induce photo-chemical reactions involving riboflavin degradation (Grant-Preece et al., 2017b). The degradation of this vitamin is responsible for the so-called *goût de lumière*, also known as Light-Struck Taste (LST), leading to the formation of volatile compounds which affect wine flavor (Maujean and Seguin, 1983; Haye et al., 1977). The LST is a complex process, which gives heavy off-odors such as those of rotten eggs and cooked cabbage due to the generation of volatile sulfur species (Dozon and Noble, 1989). The formation of these volatile compounds in wines exposed to light is related to the photoinduced oxidation of sulfur containing amino acids (methionine, cysteine) by riboflavin (Fracassetti et al., 2019). Thus, in order to extend the shelf life of rosé wines, the interactions of these wine components with light must be avoided, either

* Corresponding author. Departamento de Ciencias, Universidad Pública de Navarra, Campus Arrosadía s/n, 31006, Pamplona, Spain.
** Corresponding author. Asociación de la Industria Navarra, Carretera Pamplona 1, 31191, Cordovilla, Spain.
E-mail addresses: ealmandoz@ain.es (E. Almandoz), irene.esparza@unavarra.es (I. Esparza).

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by modifying the winemaking processes or by using containers capable of filtering this radiation.

The photodegradation processes in rosé wine occur over time (a few months of exposure to radiation). This means that the search for photoprotection strategies requires studies to be carried out over long periods of time, which therefore compromises their feasibility. For this reason, performing accelerated degradation assays emerges as a key tool to analyze different properties of wines and predict some defects with significant commercial impact. Once the marker parameters of wine deterioration due to radiation have been identified, it will be possible to test different physical and chemical protection strategies for rosé wines against light. A fair number of studies have been focused on the use of different methods to induce wine accelerated degradation by means of different irradiation sources: xenon lamps, mercury arc radiation, fluorescent light or UV radiation, among others (Maury et al., 2010). However, most of these irradiation assays are not accurate in reproducing real storage conditions, as they analyze only a specific range of the solar spectrum or involve temperatures above normal storage conditions (Pati et al., 2019; Grant-Preece et al., 2017a; Lan et al., 2021). Furthermore, some of these tests require medium-long exposure times (several weeks), which are generally impractical for specific research purposes (Maury et al., 2010).

In this work, a device capable of accelerating the degradation of rosé wines using W lamps radiation for short times of exposure has been developed. The results obtained after the treatment of rosé wines bottled in bottles with different light-filtering power were compared with those from the same wine stored in darkness. Thus, the study aims to determine the effect of UV–Vis radiation on rosé wines quality over time, also analyzing the protective role of the bottle color, and identifying the key parameters which determine the loss of rosé wines properties. The choice of rosé wines for this study was motivated by the very limited number of papers in the literature concerning these wines compared to white or red ones, and by the fact that they are generally marketed in Flint bottles despite their high susceptibility to LST. In addition, the consumption of rosé wines worldwide in recent years has grown very significantly, and hence the interest in improving their shelf life as well as their quality.

2. Materials and methods

2.1. Rosé wine making and bottling

Rosé wine was made with Grenache, Merlot and Cabernet Sauvignon varieties of the 2019 vintage in the OCHOA cellar (Olite, Spain). The must of each variety was obtained by separating the free run juice after a short maceration (4–6 h), and then each must was vinified separately. Alcoholic fermentations took place at controlled temperature (16–17 °C) by wild yeast. After the fermentation, wines were racked to different tanks, where remained for a month in contact with fine lees. Subsequently, the wines obtained from the different grape varieties were mixed in a 2:2:1 ratio in a large capacity tank and the wine thus obtained was clarified with bentonite. Before bottling, tartaric stabilization was carried out at 4 °C. The sulfur levels during the winemaking process were reduced to a minimum so as not to interfere with the color of the wine. Nitrogen was used to reduce the amount of oxygen consumed by the wine during the bottling process. The initial value of sulfites was 20 ppm. Standard 0.75 L Antique Green and Flint glass bottles were used for this research work.

2.2. Intensive irradiation equipment set-up

The light source used in the irradiation experiments were tungsten filament and high-pressure lamps (ULTRA-VITALUX®, Osram, Munich, Germany). 24 lamps were used, arranged in 6 × 4 rows and placed at a distance of 250 mm between them, and 500 mm above the bottles (Fig. 1). The total irradiation at the working distance was approximately

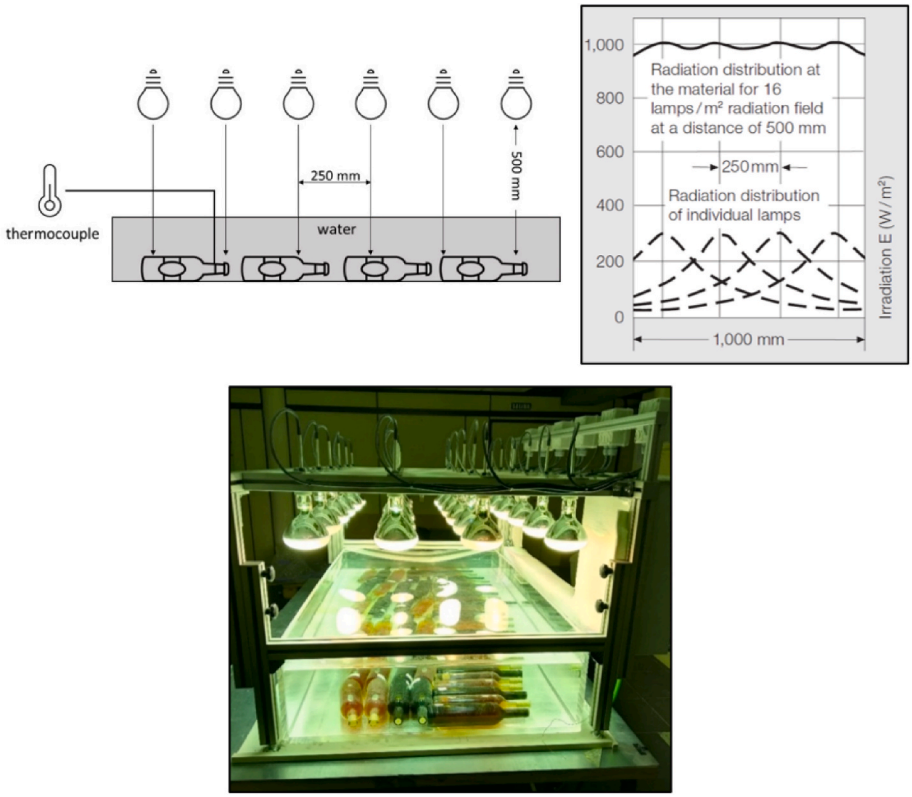

Fig. 1. Schematic illustration of the large-scale wine irradiation arrangement, test field with 24 ULTRA-VITALUX® lamps according to manufacturer's data and set-up of the tests.

1000 W/m^2, equivalent to irradiation of natural sunlight on the earth's surface with the sun is in zenith position (detailed information by the provider).

During the assays, wine bottles were placed inside a cool water bath to keep the wine temperature below 26 °C. The lid of the box was made of UV-transparent polymethylmethacrylate (PMMA). Thermocouple allowed measurement of the bottle surface temperature during the course of the irradiation. The UV–Vis radiation that the bottles received was controlled by a ST-510 UV meter (Graigar Technology, Shenzhen, China).

2.3. Experimental design

The experimental design proposed in this research addresses a study to analyze the evolution of a rosé wine subjected to accelerated degradation conditions in Flint and Antique Green glass bottles (0.75 L).

All rosé wine bottles were kept at 19 °C under darkness conditions for 6 months until all the oxygen was consumed, to avoid its interference during the irradiation tests. Then, three bottles of each type were subjected to 16, 32 and 64 h of irradiation. Every 8 h, each bottle was moved out of its position and rotated to ensure that all the bottles receive the same irradiation, thus preserving the homogeneity of the assays. Samples taken after 16 and 32 h were kept in total darkness at room temperature until the completion of the photodegradation assay, and then all the samples were analyzed at the same time. The untreated wine was kept in darkness throughout the study as control.

2.4. Determination of the antioxidant capacity and total phenolic profile (total polyphenol, flavonoid and anthocyanin content)

The total anthocyanin content was determined by a spectrophotometric validate pH differential method (Lee et al., 2005). Samples were diluted in a 1:4 ratio with two buffer solutions: one of pH 1 (0.025 M potassium chloride buffer) and another one of pH 4.5 (0.4 M sodium acetate buffer). Then the absorbance was measured at two wavelengths (520 nm and 720 nm) for each pH. Rosé wine samples were previously diluted 1:4 with methanol. Results were calculated through the following expression:

$$Total\ anthocyanin\ content\ (expressed\ as\ malvidin-3\text{-}glucoside\ equivalents, mg/L) = \frac{A \cdot MW \cdot DF \cdot 10^3}{\varepsilon \cdot P},$$

where $A = (A_{520\ nm} - A_{700\ nm})$ *at* pH *1.0* $- (A_{520\ nm} - A_{700\ nm})$ *at* pH *4.5; MW = molecular weight of malvidin-3-glucoside; DF = Dilution factor (4);* $\mathcal{E}$ *= extinction coefficient, in* $L \cdot mol^{-1} \cdot cm^{-1}$*, for malvidin-3-glucoside (28000); P = Path length in cm (*1 cm*).*

The total flavonoid content of the samples was measured by the colorimetric method of aluminum chloride according to Chandra et al. (2014). Quercetin was used as standard for the calibration curve and the results were expressed as mg of quercetin equivalents/L. For the analysis, samples of rosé wine were diluted 1:2 in methanol.

The total polyphenol content of the rosé wines was measured using the Folin-Ciocalteu method (Singleton et al., 1999). Gallic acid was used as standard for the calibration curve and the results were expressed as mg of gallic acid equivalents/L. For the analysis, samples of rosé wine were diluted 1:2 in methanol.

The antioxidant capacity was determined by three different methods: ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay, DPPH (2,2-diphenyl-1-pycrilhydracyl) radical scavenging assay and FRAP (Ferric Ion Reducing Antioxidant Power). All methods were performed according to Esparza et al. (2020) but diluting 1:4 the rosé wine samples with methanol. All the antioxidant capacity determinations were made in triplicate, and the results of antioxidant capacity were expressed as mmol of Trolox equivalents/L.

All the reagents used in these analyses were from Sigma-Aldrich (Madrid, Spain), and all the spectrophotometric analyses were performed with a UV/Vis spectrometer (Jenway 7315, Staffordshire, UK). The calibration parameters are compiled in Table S1 of the Supplementary Material.

2.5. Evaluation of wine chromatic properties

Absorbance curves were studied collecting data from 800 to 250 nm wavelength and using the standard measuring method of a PerkinElmer Lambda 950 UV/VIS/NIR spectrophotometer (Waltham, Massachusetts, USA). Distilled water was set as Zero and dark stored wines as reference. Curves at different exposure times showed a global idea of the behavior and evolution of the different wines. Values at 420 nm, 520 nm and 620 nm were used to calculate the classic color parameters defined by Glories (1984): color intensity (CI), hue and color composition (% yellow, % red, % blue). The CIELab color coordinates of the wines (L*, a*, b*) were also determined with the same spectrophotometer through the reflectance data. The PerkinElmer Lambda 950 UV/VIS/NIR spectrophotometer converts automatically the collected reflectance of samples into their CIELab color coordinates.

In order to identify and categorize perceptible changes in the color of the wines, the ΔE values based on the Euclidean distance between two points in the CIELab space was calculated (Mokrzycki and Tatol, 2011; CIE, 2004).

2.6. Determination of individual phenolic compounds and riboflavin content

The evolution of individual phenolic compounds in wines was analyzed by HPLC-DAD, using a Waters chromatograph equipped with two 515 pumps, a U6K injector and a photodiode array 996 detector (Waters Div., Milford, Massachusetts, USA). For that, wine samples were centrifuged at 5000 rpm for 10 min (Ohaus FrontierTM FC5513, Nänikon, Switzerland) to remove suspended particles and filtered through a 0.45 μm PTFE syringe filter. Control and data analyses were carried out using Empower 2.0 software. A ZORBAX Eclipse Plus C18 column (4.6 × 250 mm, 5 μm, Agilent, Santa Clara, California) was used. Mobile phases were: A (water: acetic acid 98: 2) and B (methanol: acetic acid 98: 2). The gradient program of phase A was (t in min; % of phase A): (0; 95%), (0.5, 95%), (10, 90%), (25, 80%), (35, 70%), (50, 50%), (30, 70%), (10, 90%), (5, 95%) and (95, 5%). The column temperature was kept at 35 °C and the flow rate was 1 mL/min. The injection volume was 40 μL. Samples were injected after filtration and without prior dilution. Peaks corresponding to the phenolic compounds were identified comparing both their retention time and UV–Vis spectrum with those of commercial standards. Prior to the quantification, different standards of phenolic compounds were injected in order to determine which ones were present in the rosé wines. The compounds that were injected were: gallic acid, caftaric acid, caffeic acid, syringic acid, chlorogenic acid, p-coumaric acid, catechin, epicatechin, quercetin, quercetin-3-glucoside, resveratrol, viniferin, malvidin-3-glucoside, malvidin, cyanidin-3-glucoside, procyanidin A2 and procyanidin B1. All the standards used were from the Sigma-Aldrich brand (Madrid, Spain), except malvidin-3-glucoside, which was from the Extrasynthese brand (Genay, France). To perform the calibration curves of the compounds analyzed in rosé wines, a multi-standard stock solution was prepared with all the identified phenolic compounds dissolved in methanol.

Riboflavin content was analyzed simultaneously with individual phenolic compounds, but in this case, a W474 Fluorescence detector (Waters Div., Milford, Massachusetts) was used. Peaks corresponding to this compound were identified comparing its retention time with the fluorometric peak of the commercial standard. The curve of calibration was obtained preparing a riboflavin (Sigma-Aldrich, Madrid, Spain) stock solution in water.

The concentration range and determination coefficient of each

calibration curve can be found in Table S1 of the Supplementary Material.

2.7. Statistical analyses

Due to the small sample size, non-parametric statistical tests were used to analyze the results obtained. The Kruskal-Wallis test was used to determine the existence of differences due to the applied treatment. In addition, the pairwise comparisons (*post-hoc* with Bonferroni correction) were applied to identify differences in pairs between the control (untreated wine) and those wines subjected to the accelerated degradation process in Flint and Antique Green bottles. On the other hand, a Pearson's correlation matrix analysis was performed with all data obtained throughout the study (antioxidant activity, total and individual phenolic content and chromatic parameters). All data processing was conducted by using the statistical package IBM* SPSS* Statistics version 27 (IBM Corporation, Armonk, NY, USA).

3. Results and discussion

3.1. Phenolic composition and antioxidant activity of wines

The phenolic composition of wines was estimated through the determination of total anthocyanin content (TAC), total flavonoid content (TFC), and total phenolic content (TPC). Anthocyanins, responsible for the color of rosé wines, are very unstable and sensitive to changes in pH, temperature and light, so it is very important to know how their total content evolves over the radiation exposure. Table 1 shows its total concentration in the rosé wines during the accelerated degradation process in both types of bottle, as well as in the control wine. The TAC decreased considerably after 16 h of irradiation treatment in wines contained in both types of glass bottles, although the decrease was somewhat higher in the wines irradiated in Flint bottles. Later, TAC hardly changed in wines in Antique Green bottles until the end of the treatment (64 h). However, in rosé wines treated in Flint bottles, this concentration continued to decrease, so that after 64 h of treatment, the loss of anthocyanins was greater than 70% respect to the untreated wine. Statistical analyses showed the existence of significant differences in TAC between the control wine and wines subjected to accelerated degradation in different types of glass bottles. Likewise, significant differences were also observed between wines treated in Antique Green bottles and wines treated in Flint bottles (Table 1).

It is well known that light and temperature accelerate the loss of anthocyanins in both musts and wines (Benucci, 2020; Muche et al., 2018; Stávek et al., 2012). Stávek et al. (2012) studied the effect of both factors on the anthocyanin profile of rosé wines during 582 days and concluded that the effect of irradiation on the pigments was less important than the effect of high temperatures (45 °C). In our case, the decrease in anthocyanins observed after 16 h of treatment in all the wines could be due to the action of both variables, always bearing in mind that our setpoint temperature was much lower. However, the influence of the UV radiation was decisive for the lower TAC found in the wines irradiated in Flint bottles, regardless of the duration of the accelerated degradation treatment. The irradiation of anthocyanins in model beverages have been found to cause the anthocyanin autoxidation (Gérard et al., 2019). Irradiation produces the transition of these natural pigments to an excited state, giving rise to the formation of radicals. Subsequently, these radicals react with oxygen forming highly reactive peroxyl radicals, which in turn degrade anthocyanins (anthocyanin autoxidation). Dangles and Fenger (2018) have suggested that the autoxidation phenomenon, together with hydrolysis reactions, are the most common pathways for anthocyanin degradation.

Regarding the content of TFC and TPC of the rosé wines, a decrease was also observed in all the samples subjected to accelerated degradation for 16 h, but subsequently the levels of these parameters hardly changed (Table 1). The decrease in TFC values observed at 16 h was somewhat higher in the wines irradiated in Flint bottles, while the reduction in TPC was very similar in all treated wines. Therefore, unlike what has been observed for TAC and TFC levels, no significant differences were found in TPC between irradiated wines in different types of bottle (Table 1). Therefore, light did not exert a significant impact on the total content of phenolic compounds in the rosé wine. This agrees with Benucci (2020), who also found that both temperature (30 °C) and UV radiation had a greater influence on TAC than on TPC of sparkling rosé wines subjected to these conditions in Antique Green bottles.

Table 2 shows the antioxidant capacity of the wines in Flint and Antique Green bottles throughout the treatment with UV–Vis light in the

Table 1
Total anthocyanin content (TAC), total flavonoid content (TFC) and total phenolic content (TPC) of rosé wines subjected to accelerated degradation in Flint and Antique Green glass bottles and of control wines. Statistical analysis by Kruskal-Wallis and pairwise comparisons (adjusted with Bonferroni correction) *post-hoc* tests.

Samples	Irradiation Time **(h)**	TAC[a]	TFC[b]	TPC[c]
Control	–	5.4 ± 0.0	11.5 ± 0.1	220.1 ± 4.4
Flint bottle	16	2.2 ± 0.1	10.3 ± 0.3	200.7 ± 4.8
	32	1.9 ± 0.1	10.3 ± 0.1	201.1 ± 5.5
	64	1.4 ± 0.1	10.5 ± 0.2	199.9 ± 3.8
Antique Green bottle	16	2.9 ± 0.1	10.7 ± 0.2	202.4 ± 4.0
	32	2.7 ± 0.1	10.8 ± 0.2	200.2 ± 3.1
	64	2.6 ± 0.1	11.0 ± 0.1	202.6 ± 3.3
Kruskal Wallis test (H)		43.295[d]	39.588[d]	24.491[d]
post-hoc test				
Control – Flint bottle		−41.850[d]	−40.950[d]	−33.133[d]
Control – Antique Green bottle		−18.667[e]	−19.667[e]	−28.352[d]
Flint bottle – Antique Green bottle		−23.183[d]	−21.283[d]	−4.781

[a] as mg/L malvidin-3-glucoside.
[b] as mg/L quercetin.
[c] as mg/L gallic acid.
[d] Confidence level >99.9%.
[e] confidence level >95%.

Table 2
Antioxidant capacity of rosé wines subjected to accelerated degradation in Flint and Antique Green glass bottles and of control wines. Statistical analysis by Kruskal-Wallis and pairwise comparisons (adjusted with Bonferroni correction) post-hoc tests.

Samples	Irradiation Time **(h)**	Antioxidant capacity **(mM Trolox)**		
		ABTS	DPPH	FRAP
Control	–	1.84 ± 0.01	0.91 ± 0.01	1.72 ± 0.01
Flint bottle	16	1.70 ± 0.07	0.76 ± 0.02	1.49 ± 0.03
	32	1.59 ± 0.04	0.84 ± 0.02	1.51 ± 0.03
	64	1.59 ± 0.04	0.85 ± 0.02	1.52 ± 0.01
Antique Green bottle	16	1.60 ± 0.04	0.85 ± 0.02	1.48 ± 0.03
	32	1.61 ± 0.04	0.85 ± 0.01	1.53 ± 0.01
	64	1.57 ± 0.02	0.83 ± 0.01	1.52 ± 0.03
Kruskal - Wallis test (H)		24.920[a]	23.852[a]	23.531[a]
post-hoc test				
Control – Flint bottle		−27.967[a]	−32.267[a]	−30.917[a]
Control – Antique Green bottle		−34.093[a]	−29.315[a]	−30.815[a]
Flint bottle – Antique Green bottle		6.126	−2.952	−0.102

**confidence level >95%.
[a] Confidence level >99.9%.

irradiation equipment. The antioxidant capacity was determined using three different methods (ABTS, DPPH, FRAP), since there is still no consensus on which of them is the most suitable for determining the antioxidant capacity of foods (Huang et al., 2005). The antioxidant capacity decreased in all the wines subjected to the irradiation treatment, regardless of the type of bottle and the method used in the determination. In addition, for all the methods used, significant differences were found in the antioxidant capacity between the control wine and the wines subjected to accelerated degradation in different glass bottles. However, there were no differences between the wines subjected to this treatment in Antique Green bottles and the wines treated in Flint bottles (Table 2). Therefore, the evolution of the antioxidant capacity of the wines treated in different types of bottle was similar to the evolution of their TPC levels throughout the irradiation treatment (Table 1).

Several works have correlated the antioxidant capacity of wine samples with their TPC. However, the contribution of each phenolic compound to the antioxidant activity of wines is different, so the antioxidant activity of wine will depend, to a large extent, on its phenolic profile (Luchian et al., 2018; Di Majo et al., 2008). The antioxidant activity of wines seems to be largely influenced by the total level of phenolic, flavonoid and flavanol compounds, with a minor influence of total anthocyanin content (Li et al., 2009; Di Majo et al., 2008). In fact, malvidin-3-glucoside, the most abundant anthocyanin in wine, is one of the anthocyanins that, due to its chemical structure, has lower antioxidant capacity (Castañeda-Ovando et al., 2009). This could explain why the significant decrease observed in TAC in rosé wines after the irradiation treatment did not have a direct impact on their antioxidant capacity. In fact, the decrease in the antioxidant capacity of the wines throughout the irradiation was much lower than that of the concentration of anthocyanins and correlated better with the decrease in the total flavonoids and phenolic contents.

Summarizing, and in view of all these results, the antioxidant capacity and TPC of rosé wines could not be used as photodegradation markers that help us to differentiate the light-filtering properties of the different bottles used in the irradiation treatment, since the results observed were very similar in both types of samples.

3.2. Concentration of individual phenolic compounds and riboflavin in wines

Fig. 2 shows the concentration of the phenolic compounds identified in untreated wine (control), and in wines treated in Flint and Antique Green bottles during the accelerated degradation process. The most abundant anthocyanin in grapes is malvidin-3-glucoside and, therefore, is one of the most determining components for rosé wines color. After 16 h of irradiation of the wines, the content of malvidin-3-glucoside was markedly reduced in all the treated samples (almost 50% in Flint bottled wines and around a third in Antique Green bottled wines). Subsequently, the content of this anthocyanin hardly changed in any of the wines treated with UV–Vis radiation. These results coincide with the TAC profile determined by spectrophotometric techniques (Table 1), showing that the color protection of rosé wine provided by Antique Green bottles is greater than the one provided by Flint bottles, although in no case is an effective protection. The decrease in malvidin-3-glucoside can be attributed to the same reactions described to explain the reduction in TAC. Anthocyanin autoxidation phenomena, as well as hydrolysis reactions stands out as the mechanisms responsible for this degradation, although the polymerization of monomeric anthocyanins to give rise to more stable oligomeric forms or the condensation with other phenolic compounds (Hermosín Gutiérrez et al., 2005), should not be ruled out either. At this point, it is important to highlight that these wine compounds are not directly oxidized by molecular oxygen, but instead oxidation occurs by active singlet oxygen and reactive oxygen species (ROS) that are produced by the gradual addition of a single electron to triplet oxygen by UV radiation or transition metals (Waterhouse et al., 2016). The differences among all the samples were clearly reflected in the results of the Kruskal-Wallis test (see Table S2 of the Supplementary Material), indicating again the better filtering power of the Antique Green bottles.

In addition to malvidin-3-glucoside, three phenolic acids were identified in the rosé wines analyzed. These acids also showed a significant reduction after 16 h of irradiation in the wines (Fig. 2), but different behaviors were observed in each of the cases depending on the type of bottle. The first difference to note is that gallic acid showed the same pattern in wines treated in different types of bottle, while caffeic and *p*-coumaric acids decreased to a greater extent in wines irradiated in

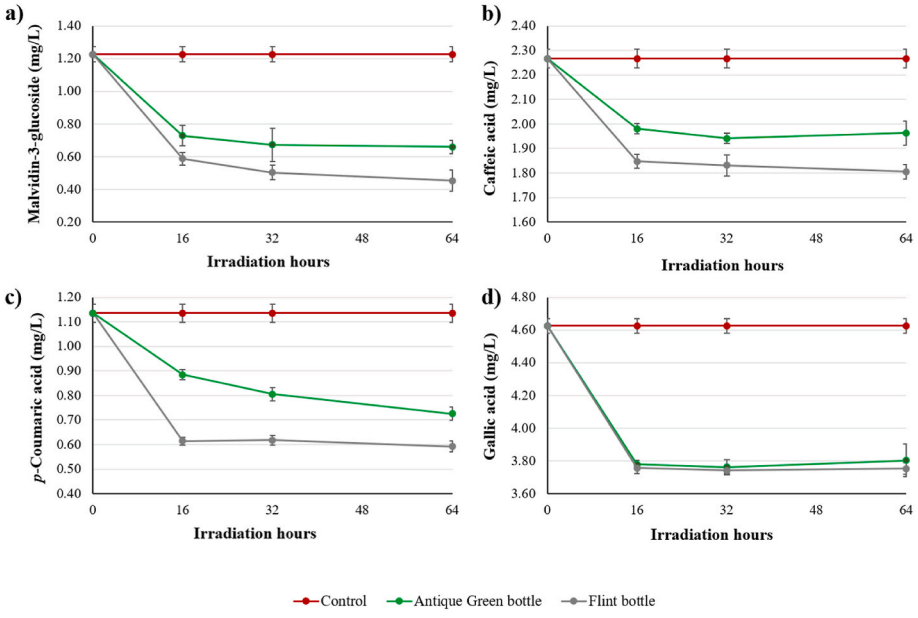

Fig. 2. Concentration of malvidin-3-glucoside (a), caffeic acid (b), *p*-coumaric acid (c) and gallic acid (d) in rosé wines subjected to accelerated degradation in Flint and Antique Green glass bottles and in control wines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Flint bottles than in those irradiated in Antique Green bottles. Moreover, *p*-coumaric acid decreased progressively throughout the accelerated degradation process in the wines treated in Antique Green bottles, unlike the rest of the phenolic compounds, whose concentration hardly changed after the reduction observed at 16 h of treatment both in Flint bottles and in Antique Green bottles. Statistical analyses showed significant differences between the different treatments applied in all cases, except in the content of gallic acid, which was very similar in the wines treated in Flint bottles as in those treated in Antique Green bottles (Table S2 of the Supplementary Material).

Esparza et al. (2020) analyzed the stability of different phenolic compounds present in Mazuelo grape stem extracts under different light (transparent and amber vials) and temperature conditions (25 and 45 °C) over 6 months. Gallic acid turned out to be one of the most stable compounds to these adverse conditions throughout the assay, since its concentration hardly showed variations. This previous result, together with the fact that in the present work both types of sample showed the same decrease (around 20%) in the concentration of this phenolic acid after 16 h of irradiation, could indicate that light is not the only factor responsible for the decrease of this compound concentration. On the other hand, thanks to its high antioxidant capacity due to the presence of three hydroxyl groups in the phenolic ring (Sroka and Cisowski, 2003), gallic acid could act as a powerful antioxidant and react with the radicals formed in rosé wine by the action of UV–Vis light during the first hours of treatment. In this way, gallic acid could reduce the anthocyanins autoxidation produced by the action of light, acting as quencher or being excited instead of the anthocyanins (Gérard et al., 2019; Roidoung et al., 2016).

Regarding the decrease in the content of hydroxycinnamic acids in rosé wines during treatment, it should be noted that these acids also have an important antioxidant activity, although less than that of gallic acid due to the lower number of hydroxyl groups and their position in relation to the carboxyl group (Rice-Evans et al., 1996; Robards et al., 1999). Therefore, caffeic and *p*-coumaric acids could also contribute to a lower autoxidation of anthocyanins by the action of light in wines treated in Antique Green bottles. On the other hand, there are some studies on the photodegradation of these phenolic compounds. Le Person et al. (2013) studied the photodegradation of caffeic acid in aqueous solutions with a mercury–xenon lamp, and proposed three different photodegradation pathways: the formation of vinylcatechol, which appears to be the major pathway; the photo-isomerization and subsequent intramolecular cyclization to give rise to esculetin; and the formation of protocatechuic acid, a minor pathway. However, the photodegradation of *p*-coumaric acid by the sunlight requires the presence of a photosensitizer to absorb the radiation and, thus, act as a catalyst in the oxidation of this acid (Amat et al., 1999). In this regard, riboflavin is a possible photosensitizer present in the rosé wines analyzed in this study, and which could trigger the photodegradation of *p*-coumaric acid as well as other phenolic compounds in wine. In Fig. 3 the concentration of riboflavin both in the control untreated wine and in those subjected to accelerated degradation is shown. After 16 h of irradiation, very low

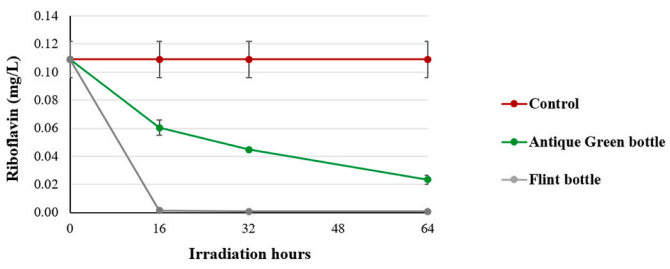

Fig. 3. Concentration riboflavin in rosé wines subjected to accelerated degradation in Flint and Antique Green glass bottles and in control wine (untreated). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

levels of riboflavin were already detected in the wines treated in Flint bottles, and after 64 h of irradiation this vitamin had completely degraded in these wines. However, in wines treated in Antique Green bottles, the content of riboflavin progressively decreased throughout the treatment, so that after 64 h of irradiation its concentration had decreased by 70% compared to the control sample (Fig. 3).

This fact could explain why the concentration of *p*-coumaric acid decreased significantly throughout all the irradiation treatment in the wines treated in Antique Green bottles while in the wines irradiated in Flint bottles no changes were observed in the concentration of this acid when vitamin B2 disappeared from the medium. Fracassetti et al. (2019) developed a fluorescent light-based photodegradation equipment to study the riboflavin degradation in model wines. These authors concluded that, in a model wine without the presence of other compounds, this vitamin was completely lost from the wine after just 2 h of exposure in transparent bottles. The irradiation source used by these Italian researchers was different from the one used in the present work and, in addition, they carried out the assays in model wine without interfering substances. Even so, it seems evident that the first sampling and analysis time selected in the present work (16 h) was too long, especially for wines irradiated in Flint bottles.

The statistical analyses showed significant differences both between the control untreated wine and those subjected to the accelerated degradation process, as well as between the wines treated in different types of bottle (see Table S2 of the Supplementary Material). Therefore, the riboflavin content is also a good indicator to screen during photodegradation assays that pursue to improve the light-filtering properties of Flint bottles, commonly used to market rosé wines. Moreover, the photoprotective effect of the Antique Green bottle compared to the Flint bottle on the riboflavin content in rosé wine has been proven.

3.3. Color parameters of wines

The classic color parameters (color intensity, hue, yellow, red and blue components, and brightness) allow dimensioning of the differences between the wines subjected to different treatments. Fig. 4 shows the evolution of color intensity (CI) and hue of the wines analyzed in this work, as well as the result of the statistical analyses of these parameters. Wines subjected to UV–Vis radiation showed decreases in CI values, this effect being greater in wines irradiated in Flint bottles than in wines treated in Antique Green bottles (Fig. 4a). The most important reduction compared to the control wine (7% in Antique Green wines, 16% in Flint wines) was observed after the first 16 h of accelerated degradation treatment in both types of samples, although in the wines treated in Flint bottles, a progressive loss of CI was observed throughout the treatment, reducing its value by more than 30% compared to the control wine. Another wine color parameter to consider is the hue, which indicates the relative importance of the yellow component with respect to the red one (Puértolas et al., 2010). The inverse of this parameter (Absorbance at 520 nm/Absorbance at 420 nm) is also known as browning index (Dorris et al., 2018). Wines treated with UV–Vis radiation reached higher hue values than the control one (Fig. 4b), contrary to what occurred with the CI, what means that a color shift towards orange tones was produced. This agrees with both the hypochromic and the hypsochromic effect observed in Fig. S1 (Supplementary Material). After the first 16 h of treatment, the hue of the wines in Antique Green bottles was similar to that of the wine kept in Flint bottles. However, subsequently, the hue of the wines in Antique Green bottle remained constant while that of the wines in Flint bottle continued to increase progressively until the end of the treatment. Statistical tests on CI and hue data confirmed both the differences due to the irradiation treatment and the differences due to the type of bottle used (Fig. 4c).

The percentages of yellow, red and blue components, together with the brightness of the wines (Table 3) are also in agreement with the hypsochromic effect previously described (Fig. S1 of the Supplementary Material). In wines treated with UV–Vis radiation in Flint bottles, a

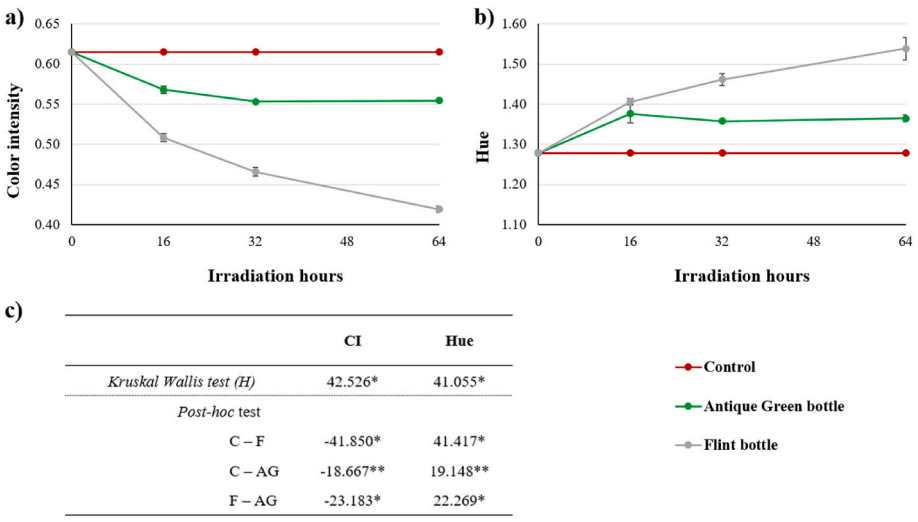

Fig. 4. Color intensity (a) and hue (b) of rosé wines subjected to accelerated degradation in Flint and Antique Green glass bottles and of control wine. Statistical analysis by Kruskal-Wallis and pairwise comparisons (adjusted with Bonferroni correction) *post-hoc* tests (c): C, Control; F, Flint glass bottle; AG, Antique Green bottle. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Color components (yellow, red and blue) and brightness of rosé wines subjected to accelerated degradation in Flint and Antique Green glass bottles and of control wine. Statistical analysis by Kruskal-Wallis and pairwise comparisons (adjusted with Bonferroni correction) *post-hoc* tests.

Samples	Time (h)	Yellow (%)	Red (%)	Blue (%)	Brightness
Control	–	52.02 ± 0.02	40.69 ± 0.02	7.28 ± 0.04	27.12 ± 0.05
Flint bottle	16	54.17 ± 0.24	38.53 ± 0.14	7.30 ± 0.27	20.22 ± 0.46
	32	55.40 ± 0.24	37.90 ± 0.23	6.70 ± 0.08	18.07 ± 0.79
	64	56.78 ± 0.46	36.91 ± 0.38	6.32 ± 0.11	14.51 ± 1.39
Antique Green bottle	16	53.54 ± 0.58	38.90 ± 0.31	7.56 ± 0.48	21.45 ± 1.02
	32	53.20 ± 0.07	39.15 ± 0.04	7.62 ± 0.03	22.40 ± 0.16
	64	53.27 ± 0.24	39.03 ± 0.13	7.70 ± 0.22	21.90 ± 0.41
Kruskal Wallis test (H)		40.944[a]	41.055[a]	34.406[a]	41.055[a]
post-hoc test					
Control – Flint bottle		41.383[a]	−41.417[a]	−13.350	−41.417[a]
Control – Antique Green bottle		19.185[b]	−19.148[b]	17.815[b]	−19.148[b]
Flint bottle – Antique Green bottle		22.198[a]	−22.269[a]	−31.165[a]	−22.269[a]

[a] Confidence level >99.9%.
[b] confidence level >95%.

progressive increase in the yellow component was observed from the beginning of the treatment, simultaneously and parallel to the decrease in the red component and, consequently, in red brightness. The reduction of the red component of the wines color, as well as of the red brightness, is mainly due to the loss of anthocyanins previously reported and coincides with the results found by Benucci (2020) after 9 months of UV-irradiation of rosé sparkling wines in Antique Green bottles.

Regarding the blue component of the wines subjected to irradiation treatment, no changes were observed in the first 16 h of light exposure, regardless of the type of bottle. But later, in the Flint bottled wines, the blue component decreased slightly while in the Antique Green bottled wines, it increased slightly. In recent years, new and more stable

Table 4
CIELab chromatic coordinates of rosé wines subjected to accelerated degradation in Flint and Antique Green glass bottles and of control wine. Statistical analysis by Kruskal-Wallis and pairwise comparisons (adjusted with Bonferroni correction) *post-hoc* tests.

Samples	Time (h)	L[a]	a[a]	b[a]
Control	–	85.9 ± 0.1	13.2 ± 0.1	14.1 ± 0.1
Flint bottle	16	88.7 ± 0.2	9.6 ± 0.0	13.9 ± 0.2
	32	89.8 ± 0.1	8.6 ± 0.0	13.6 ± 0.1
	64	91.2 ± 0.0	7.3 ± 0.1	13.1 ± 0.0
Antique Green bottle	16	87.4 ± 0.1	10.9 ± 0.0	14.5 ± 0.0
	32	87.6 ± 0.0	10.8 ± 0.1	14.2 ± 0.0
	64	87.7 ± 0.1	10.6 ± 0.0	14.3 ± 0.2
Kruskal Wallis test (H)		42.531[a]	42.533[a]	50.536[a]
post-hoc test				
Control – Flint bottle		41.850[a]	−41.850[a]	−19.500[b]
Control– Antique Green bottle		18.667[b]	−18.667[b]	18.259[b]
Flint bottle – Antique Green bottle		23.183[a]	−23.183[a]	−37.759[a]

[a] Confidence level >99.9%.
[b] confidence level >95%.

pigments derived from anthocyanins called pyranoanthocyanins have been identified in red wines, (Fulcrand et al., 1998; Hayasaka and Asenstorfer, 2002; Mateus et al., 2005; Schwarz et al., 2003). Malvidin-3-glucoside-based pyranoanthocyanins show color at all pH values and are more stable than free anthocyanins in long-term storage (Sun et al., 2020). Some of these pigments, specifically portisins, and pyranoanthocyanin dimers have bluish coloration (Zhang et al., 2022). Moreover, some anthocyanin-flavanol complexes only linked by inter-flavan bond (B-type complexes) also display violet tones (Salas et al., 2003). Antique Green bottles could protect this type of copigments to a greater extent than Flint bottles, thus explaining the differences observed in the blue component of the rosé wines studied. With the data available in this work, it is not possible to confirm these hypotheses, so additional studies would be necessary to verify the stability of these anthocyanin-derived pigments to the different wavelengths of radiation passing through the two types of glass analyzed.

To define the color of the wines more precisely, the chromatic coordinates L*, a* and b* of the CIELab space were also determined for the control wine and for the wines subjected to accelerated degradation in Flint and Antique Green bottles (see Table 4). After 64 h of irradiation, the clarity (L*) increased slightly in both types of wine, being this effect

more pronounced for the wines treated in Flint bottles. This parameter is directly related to the visual sensation of luminosity, and their values can range between 0 and 100, so that a value of 0 means black, while a value of 100 means colorless (OIV-MA-AS2-11, 2006). Therefore, the irradiation process induced a significant loss of color, which was more pronounced in the wines treated in Flint bottles. Pearson's correlation diagram (see Table S3 of the Supplementary Material) shows that there exists an inverse correlation between L* and TAC values (−0.902**). This good correlation could mean that the main cause of color loss during the irradiation process is due to anthocyanin degradation. In addition, a very high inverse correlation was also found between L* and CI values (−0.995**), which agrees with the results found by other authors in red wines and grapes (Esparza et al., 2006; Lago-Vanzela et al., 2014; Liang et al., 2011).

The two remaining coordinate axes correspond to a* and b*, which form a plane perpendicular to the clarity and represent the red to green and the yellow to blue color variations, respectively (a*>0: red; a*<0: green; b*>0: yellow; b*<0: blue). In the present study, the b* coordinate hardly changed in rosé wines treated in Flint bottles, while it showed a slight increase in the wines in Antique Green bottle, which is correlated with the blue and yellow components of the wines (see Fig. S2 and Table S3 of the Supplementary Material). However, neither the b* values, nor the classical blue or yellow color components correlate with phenolic compounds. On the other hand, a* coordinate decreased significantly in both types of wine, although to a greater extent in wines treated in Flint bottles. These results mean that the irradiation induced a loss of red color, which agrees with the decrease in red color component and brightness previously described (Table 3). This was also confirmed by the high Pearson correlation value found between a* and % red and brightness values (0.983** and 0.982**, respectively) from the different wines analyzed (Table S3 of the Supplementary Material). In addition, a high correlation was also observed between these chromatic parameters and the total anthocyanin content (0,949**, 0,941** for a* and % red, respectively) or the individual anthocyanin malvidin-3-glucoside (0,927**, and 0,920** for a* and % red, respectively). These results agree with previous studies that have also related a* coordinate with the contribution of anthocyanins to red color component both in red (Esparza et al., 2009) and rosé wines (Stávek et al., 2012).

In view of the above, it can be concluded that both classical and CIELab color parameters were altered by accelerated photodegradation processes, and could therefore be used as markers of photodegradation. However, the relative percentages of variation of L* values (from 1.7% to 6.2% of increase) are lower than those of their equivalent classical parameter CI (from 0 to 31.1% of decrease), while a* values presented higher relative percentage of variation (from 17% to 45% of decrease) than the % of red color (from 4% to 9% of decrease). In the case of b* and % blue values, the relative percentages were similar, although slightly higher in the case of % blue variations. Thus, when evaluating the photodegradation of a rosé wine, both classic and CIELab parameters should be considered, but paying special attention to the variation of CI and a* values.

On the other hand, a surprisingly good correlation was also found between the concentration of riboflavin in rosé wines and the a* coordinate (0.928**), the TAC values (0.967**), and malvidin-3-glucoside concentration (0.957**). This means that the concentration of riboflavin shows a variation pattern similar to that of anthocyanins throughout the accelerated degradation processes, so their degradation kinetics will probably be of the same order. Further studies should be conducted to confirm such hypothesis. In any case, and regardless of the cause of this correlation, what is relevant is that both the TAC and a* parameters as well as riboflavin concentration values are equally useful as photodegradation markers of rosé wines. Among them, the measurement of a* and TAC only requires a simple spectrophotometric analysis, while that of riboflavin is not so simple, requires the use of HPLC-fluorescence, and the low concentration levels of this vitamin in rosé wines make difficult its quantification in certain samples. Therefore, with a simple spectrophotometric measurement of some parameters, such as a* and TAC, it is possible to estimate not only the photodegradation degree of a rosé wine, but also that of the riboflavin loss. Further studies will be necessary to relate these losses to the appearance of the LST flavors.

Finally, the overall colorimetric differences of the treated wines with respect to the control wine were considered in order to determine if they can be noticeable for the human eye. The perception and comparison of colors by human vision depends on both external factors and individual human characteristics, so it is necessary to use mathematical models that standardize these perceptual differences between colors. Table 5 includes the color change of the wines (ΔE) based on the Euclidean distance between two points in the CIELab space and used to establish and categorize the changes in color perception of different samples (Mokrzycki and Tatol, 2011; CIE, 2004). The variation of total color (ΔE) with respect to the control sample was manifested after the first hours of irradiation in the wines treated in different types of bottle, although it was more important in the wines treated in Flint bottles than in the wines treated in Antique Green bottles. After 32 h of accelerated degradation treatment, the Flint bottle wines already showed a different color compared to the control wine, while the Antique Green bottle wines only showed a perceptible change. According to Zhang et al. (2016), the color discrimination threshold by human eye suggested for red wines is $\Delta E = 3$. Considering this limit, the protective effect of the Antique Green bottle was even more evident since until the end of the accelerated photodegradation study, no appreciable changes in the color of the rose wines were detectable, while in the wines in Flint bottles there were clear color alterations from the first timepoint.

4. Conclusions

In the present work, an irradiation equipment capable of degrading riboflavin, phenolic compounds, color and, therefore, the quality of rosé wines in just 16 h and at controlled temperature, has been developed. Although the loss of quality of the wines due to the irradiation treatment was observed in all treated wines, the greater protection of Antique Green glass compared to Flint glass has been demonstrated, what probes the usefulness of the equipment for comparing the light-filtering properties of the different types of bottle. In this study, it was found that the most relevant parameters of rosé wines affected by the irradiation were the total anthocyanin content and color values (especially CI and a*) together with malvidin-3-glucoside and riboflavin content. Among them, the measurement of a* and TAC only requires a simple spectrophotometric analysis, while that of malvidin-3-glucoside and riboflavin is not so simple, requires the use of HPLC-DAD or HPLC-fluorescence, and their values may be below the quantification limits of the method in degraded samples. Therefore, the parameters TAC, CI and a* are preferred for the evaluation of the degree of photodegradation of a rosé wine, and can be considered as good photodegradation markers. On the other hand, the results obtained in the present work allow also

Table 5
Color change (ΔE) after 16, 32 and 64 h of accelerated degradation in Flint and Antique Green bottled rosé wines.

Samples	Time **(h)**	Color evolution	
		ΔE	Magnitude of change[a]
Control	–		
Flint bottle	16	4.6	Clear difference in color
	32	6.0	Two different colors
	64	8.0	Two different colors
Antique Green bottle	16	2.8	Noticeable
	32	3.0	Noticeable
	64	3.2	Noticeable

[a] Criteria established by Mokrzycki and Tatol (2011) for the interpretation of ΔE values.

concluding that antioxidant capacity and TPC values of rosé wines should not be considered as suitable photodegradation markers, since they do not allow differentiation between wines that have been treated in different bottles. Nevertheless, it would be necessary to monitor the evolution of all these parameters in shorter irradiation times, as well as to establish correspondences, based on the selected markers, between the irradiation times in accelerated conditions and the natural exposure to cycles of sunlight and/or fluorescent light that usually exist in markets or at home.

In any case, this work has demonstrated that the developed irradiation equipment allows to monitor in a fast and simple way the photodegradation of rosé wines, which is of great interest for the successful future development of new photoprotective strategies for rosé wines and other wines such as white or sparkling wines, usually commercialized in Flint bottles.

CRediT authorship contribution statement

Jennifer Moriones: Methodology, Formal analysis, Data curation, Writing – review & editing. **Nerea Jiménez-Moreno:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Carmen Ancín-Azpilicueta:** Conceptualization, Writing – review & editing, Funding acquisition. **Jonathan Fernández de Ara:** Conceptualization, Methodology, Writing – original draft. **Beatriz Navarcorena:** Formal analysis. **Eluxka Almandoz:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Irene Esparza:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100501.

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