Modeling color and chemical changes on normal and red heart beech wood by reflectance spectrophotometry, Fourier Transform Infrared spectroscopy and hyperspectral imaging

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Abstract

The use of beech is a key topic for Europe as it is one of the most important and abundant broad-leaf species. Physical, mechanical and esthetical features influence both the value and the usage of this wood in each commercial product. In this sense, the comprehension of the surface color modifications induced by solar irradiation is of crucial importance to define the commercial value of the beech wood. Starting from these general remarks, the aim of this work is to study the surface modifications on beech wood with and without red heartwood by different spectroscopic techniques and to obtain a modeling of the changes validated by rigorous statistical and chemometric methods together with principal component analysis. The artificial photo-irradiation of the wood samples was performed in a Solar Box. Reflectance spectrophotometry, Fourier Transform Infrared spectroscopy and hyperspectral imaging were used to assess artificial sunlight influence. The experimental data were statistically treated in order to evaluate their significance. Color monitoring allowed to find that the chromatic coordinates (L*a*b*) in normal wood and in red heartwood tended to similar values after 504 h of photo-irradiation. Fourier Transform Infrared spectroscopy showed the rate of photo-degradation of wood surface due to lignin oxidation and the statistical analysis allowed to demonstrate that red heart and normal wood have the same behavior. Concerning hyperspectral imaging (HSI), the detected spectral features were correlated to color changes in the Visible-Near Infrared (VIS-NIR) range (400-1000 nm) and to the variations of cellulose and lignin during accelerated aging in the Short-Wave Infrared (SWIR) range (1000-2500 nm). The most important result is that a correlation, validated by statistical analysis, of the color changes may be derived with the photo-degradation of wood components obtained by spectral analysis. This fact suggests the possibility to choose the reflectance spectrophotometry as a non-invasive, simple standard method to monitor the state of preservation of the wood surfaces

1. Introduction

The aim of this work is to study the surface modifications on beech wood, with and without red heartwood, by different spectroscopic techniques in order to obtain a modeling of the changes validated by rigorous statistical and chemometric methods together with principal component analysis.

The choice of beech wood was due to its wide use in Europe [1], as it is one of the most important and abundant broad-leaf species. The extent of beech forests in Europe is estimated at about 12 million hectares of which more than 550,000 in Italy [2]. In particular, beech wood from coppices in transition shows interesting qualitative characteristics, suggesting a more profitable use than firewood [3]. Coppices in transition originated from the conversion of large areas from coppice to high forest as a consequence of the progressive abandonment of rural and mountain zones and policies of ecological protection of the territory in the last century, leading forest managers to a less intensive use of forests [4], creating different situations in Europe [5-7]. In Central Italy, large areas were converted to coppice, as a result of changing the socioeconomic conditions of the last century, the gradual rise in the cost of labor and the stagnation in the market prices of firewood. In Italy, the

transitory high forests stand on about 150,000 ha [8]. In Italy, the wood sector involves about 80,000 businesses, over 500,000 work units and it is heavily dependent on foreign countries for the supply [9], as the domestic timber availability is not always appreciated due to the lack of quantitative and qualitative homogeneity. From coppices in transition to high forest, which now reached maturity and therefore are ready to renewal cuts, firewood or biomass in the form of particles are obtained, mainly for energy production [3,10,11]. However, some species are particularly valued for their technological features, valuable products also are obtained as round wood and sawn timber, despite the large amount of branched and buckled stems. Concerning the beech wood, the occurrence of red heartwood due to wounds, root dieback and dead branch constitutes a drawback. The grading rules concerning sawn timber, as EN 975-1 [12], limit the presence of red heart. For this reason, red heartwood is less appreciated [13,14] and causes loss of commercial value [15,17], influencing the yield of the production. As a consequence, the study and comprehension of beech color parameters are crucial to define the potential usages of this species for higher quality production, in order to enhance the wood chain from the forest to the processing industry.
In general, color variation of wood due to light radiation is an interesting and important topic in wood science as testified by a lot of literature papers concerning the photo-oxidation of wood surfaces [18,29]. In particular, the occurrence of red heartwood in beech is definitely a significant characteristic that often leads to debasement following classical grading rules. For this reason, the modeling of color and chemical changes on beech wood with or without red heartwood becomes important. To do this, reflectance spectrophotometry, Fourier Transform Infrared (FTIR) spectroscopy and hyperspectral imaging were applied to the two kinds of wood. The datawere correlatedto check the relationship between variation of color and of the chemical components [24].

2. Materials and methods

2.1. Sample preparation and aging

Wood samples were obtained by boards of beech harvested on Terminillo Mountain (Central Italy - Leonessa Municipality) in a coppice in transition to high forest. Information on the site and sample preparation was detailed in a previous paper [3]. All samples were artificially aged in a Model 1500E Solar Box (Erichsen Instruments). The system is equipped with a 2.5 kW xenon-arc lamp and a UV filter that cuts off the spectrum at 280 nm. The samples were exposed in the Solar Box chamber from 1 to 504 h at 550 W m^{-2}, 55 °C and the UV filter at 280 nm. The experimental conditions were chosen following the specifications supplied by Erichsen, in order to simulate the sunlight exposition. Inside the Solar Box chamber relative humidity was constant and determined by the irradiation conditions. Relative humidity was monitored by a data logger positioned inside the Solar Box.
To perform the FT-IR analysis directly on wood surface, slices with a size of 10 mm in diameter and 2 mm in thickness were obtained from the specimens of beech. The dimensions of the slices were suitable for the FT-IR diffuse reflectance accessory.

2.2. Color monitoring

After exposure for a given length of time the samples were removed from the Solar Box chamber and the color was measured using an X-Rite CA22 reflectance spectrophotometer according to the CIELAB color system. The characteristics of the color measuring instrument are the following: light source D65; standard observer 10°; fixed geometry of measurement 45°/0°; spectral range 400-700 nm; spectral resolution 10 nm; aperture size 4 mm [24,25,30-32]. The wood samples were conditioned before color measurements at 22 °C and 50% RH.
Measurements were taken at the following hour intervals: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 48, 72, 96, 120, 144, 168, 216, 312, 408 and 504 h.
Sixty color measuring points were chosen: thirty for red heartwood and thirty for normal beech wood. Three measures for each point were performed, so that one hundred and eighty measurements were obtained at each exposure time, then average values and standard deviations were calculated.

2.3. Fourier Transform Infrared spectroscopy

Infrared spectra were obtained using a Nicolet Avatar 360 Fourier transform spectrometer. For each sample 128 scans were recorded in the 4000 - 400 cm^{-1} spectral range (2500-25,000 nm) in diffuse reflection modality (DRIFT) with a resolution of 4 cm^{-1}. As background the spectrum of the KBr powder was used. Spectral data were collected with OMNIC 8.0 (Thermo Fisher Scientific Inc.) software.

FT-IR spectra were recorded at the following time intervals: 0, 6, 12, 24, 48, 72, 96, 120, 144, 168, 216, 312, 408 and 504 h.

Peak heights were measured using OMNIC software according to the method described in the literature [33].

2.4. Hyperspectral imaging

Hyperspectral analyses were carried out in two steps. A first step was addressed to analyze the degraded wood surfaces in the wavelength interval 400-1000 nm (VISeNIR). A second step was finalized to perform investigations in the wavelength interval 1000-2500 nm (SWIR).

For the first set of acquisitions (400-1000 nm), the ImSpector V10E™ (Specim, Finland) was installed on a Leica M205C™ stereomicroscope. The energizing source was constituted by a MI-150 Dolan Jenner fiber optic device equipped with a dichroic lamp. The magnification was 7.8 x, corresponding to a spatial resolution of 40 micron. The spectral resolution was 5 nm. For the second set of acquisitions (1000-2500 nm) the SISUChema XL™ (Specim, Finland) was used, equipped with a 31 mm lens allowing the acquisition of wood samples with a resolution of 300 micron/pixel. The spectral resolution was 6.3 nm. Images were acquired through scanning each investigated sample line by line.

The calibration was performed through recording black and white reference images. Certified standards were used. Black image (B) was acquired to remove the dark current effect of the camera sensor. White reference image (W) was acquired using a ceramic tile, calibrated with a NPL Spectralone specimen, in the same condition employed for the raw image acquisition. The image correction was thus performed adopting the following equation:

$$I = \frac{I_0 - B}{W - B} \times 100,$$

where I is the corrected hyperspectral image in a unit of relative reflectance (%), I0 is the original hyperspectral image, B is the black reference image (~0% reflectance) and W is the white reference image (~99.9% reflectance). All the corrected images were then used to perform the HSI-based analysis, that is to extract spectral information.

2.5. Statistical and spectral analysis

Data obtained by color measurements and FT-IR spectroscopy were analyzed with the Statistica 2010 advanced statistics software [34]. As a first step, data distribution was plotted and visually checked for normality. Differences between treatments were checked with the standard paired t-test, with ANOVA and MANOVA analysis. Post-hoc test was conducted with Tukey Honest Significant Difference (HSD) test method. Linear and non-linear regression analysis was used to develop prediction models.

Concerning HSI, spectral data analysis was carried out adopting standard chemometric methods [35-36], by the PLS_Toolbox (Version 7.8 Eigenvector Research, Inc.) running inside Matlab (Version 7.11.1, The Mathworks, Inc.). More in details, the spectra preprocessing was performed as follows: raw spectra were preliminary cut, at the beginning and at the end of the investigated wavelength

range, in order to eliminate unwanted effects due to lighting/background noise. Concerning the VIS-NIR range, it was not necessary to cut the spectra, so the entire wavelength range (400-1000 nm) was investigated. Spectra were preprocessed applying mean centering. Regarding the SWIR range, the wavelength reduction was from 256 to 240 resulting in an investigated spectral range of 1005-2500 nm. In this case the following preprocessing algorithms were applied: 1st derivative, standard normal variate (SNV) and mean centering.
Principal component analysis (PCA) is a powerful and versatile method capable of providing an overview of complex multivariate data. PCA can be used e.g. for revealing relations between variables and relations between samples (e.g. clustering), detecting outliers, finding and quantifying patterns, generating new hypotheses as well as many other things [37]. It was used to decompose the "processed" spectral data into several principal components (PCs) (linear combinations of the original spectral data) embedding the spectral variations of each collected spectral data set. According to this approach, a reduced set of factors is produced. Such a set can be used for discrimination, since it provides an accurate description of the entire dataset. The first few PCs, resulting from PCA, are generally utilized to analyze the common features among samples and their grouping: in fact, samples characterized by similar spectral signatures tend to aggregate in the score plot of the first two or three components. Spectra could be thus characterized either by the reflectance at each wavelength in the wavelength space, or by their score on each PC in the PC space. Samples characterized by similar spectra, belonging to the same class of products, are grouped in the same region of the score plot related to the first two or three PCs, whereas samples characterized by different spectral features will be clustered in other parts of this space.

3. Results and discussion

3.1. Color changes of wood samples

Wood color, expressed as L*a*b* coordinates, exhibits positives values both in normal and red heart beech, before and after the irradiation in Solar Box (Table 1). The normal wood coordinates before aging are similar to those found by other authors for Serbian beech [38] but quite different from those of Japanese beech (Fagus crenata) [39].
The red heartwood coordinates before aging are similar to those found by other authors for Serbian beech [38].
By comparing the chromatic coordinates, it can be observed that L* value before irradiation is clearly smaller in red heartwood than in normal wood whereas the values of a* and b* parameters are larger in the red heartwood so enhancing the yellow and red hue in this kind of wood. The L*a*b* changes as function of irradiation time are shown in Figure. 1. L* parameter clearly decreases in normal wood as observed for other species [25,29,31e32,40e45]. The rapid decrease of lightness on the normal wood can be attributed to photodegradation processes mostly related to the decomposition of lignin due to the chromophore groups absorbing energy, especially in the UV range of the sunlight spectrum [19-22]. The photodegradation of the extractives can also affect the decrease of L* values, as discussed by several authors [22,46-49].
In red heartwood, lightness undergoes a little decrease in the first 12 h. After this time L* coordinate increase reaching the final value of 69.45 at 504 h of exposure (Figure 1).
The values of the chromatic coordinate a* increase as function of exposure time in normal wood, the greatest changes occurring within the first 24 h of irradiation in Solar Box.
In red heartwood the values of a* exhibit little and fluctuating changes, as shown in Table 1. The values of the chromatic coordinate b* increase both in normal and red heartwood, during the irradiation times. The greatest changes, also in this case, occur within the first hours of exposure and indicate a clear yellowing of wood surface. It is interesting to note that the L*a*b* coordinates in red heartwood wood and in normal wood tend to close values after 504 h of exposure in Solar Box.
Color changes were also analyzed using ΔE^* variations as function of exposure times (Figure 2). After 48 h of exposure in Solar Box a rapid color change in normal wood was observed (see Figure

2 curve B). The value of ΔE * vary from 1.48 (after 1 h of exposure) to 16.23 (after 48 h of exposure), followed by little increases to reach the final value of 18.46 at the end of the irradiation.
On the red heartwood, ΔE * values increase during the time varying up to 10.11 (after 504 h). The greatest total color changes occur within the first 12,24 h, as can be observed in Figure 2, curve A.

Table 1: Average values of the L*a*b* coordinates at the different irradiation times for normal and red heart wood.

	Normal wood			**Red heartwood**		
Time (h)	**L***	**a***	**b***	**L***	**a***	**b***
0	82.06	4.92	17.36	66.85	10.47	21.28
1	81.06	4.68	18.43	65.89	9.63	21.26
2	80.22	5.13	19.39	65.54	9.74	21.70
3	79.58	5.80	20.72	65.45	10.18	22.61
4	78.19	6.10	21.09	64.63	9.86	22.25
5	77.21	6.37	21.80	64.46	9.76	22.55
6	76.80	6.77	22.29	64.31	9.79	22.76
7	76.15	7.14	22.78	64.12	9.87	23.02
8	75.54	7.47	23.24	63.86	10.05	23.47
9	75.13	7.99	24.19	63.86	10.35	24.24
10	74.42	8.02	24.03	63.66	10.06	23.90
11	73.74	8.23	24.28	63.69	10.00	23.87
12	73.39	8.42	24.51	63.36	10.13	24.21
24	71.90	9.96	27.88	64.44	10.67	27.23
48	70.78	9.85	27.94	65.61	9.97	27.93
72	70.16	9.76	28.21	66.57	9.70	28.66
96	69.25	9.12	27.49	66.30	8.85	27.79
120	69.44	8.99	27.60	66.89	8.71	28.39
144	69.26	9.08	27.91	67.32	8.73	28.79
168	69.51	9.97	29.95	67.73	9.47	31.02
216	68.44	9.48	29.14	67.40	8.96	30.38
312	69.18	9.69	29.90	69.07	8.86	31.22
408	69.01	9.38	29.46	68.58	8.58	31.03
504	68.99	9.49	29.57	69.45	8.49	31.05

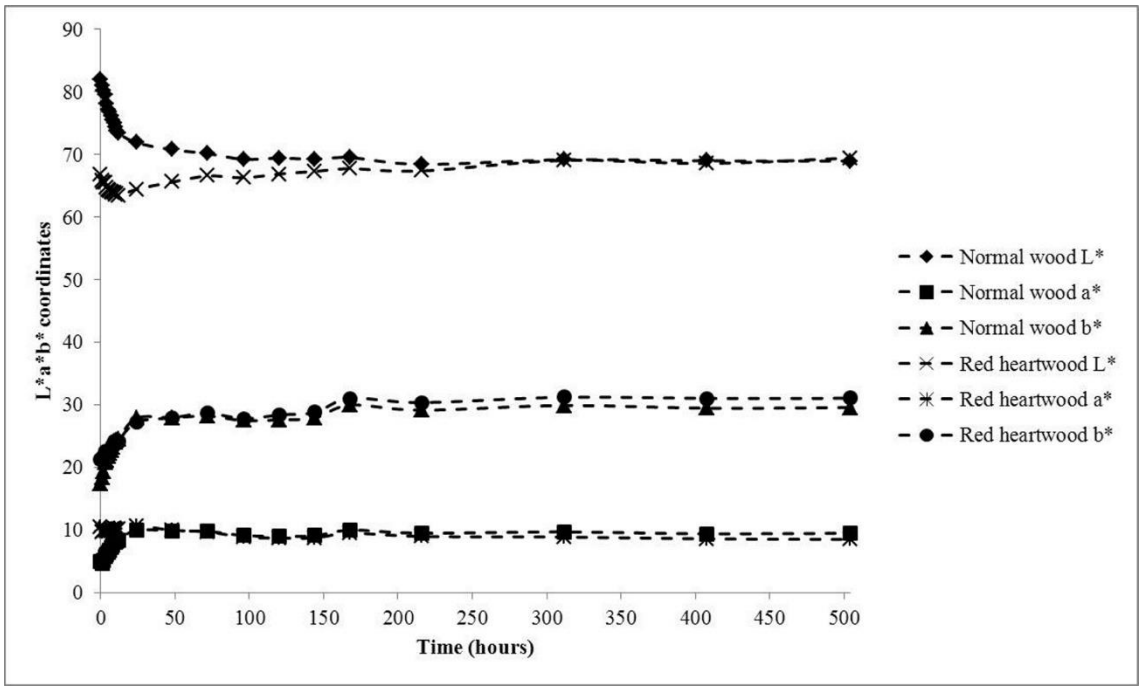


Figure 1. Changes in L*a*b* values due to exposure in Solar Box of the normal and red heart wood over 504h.

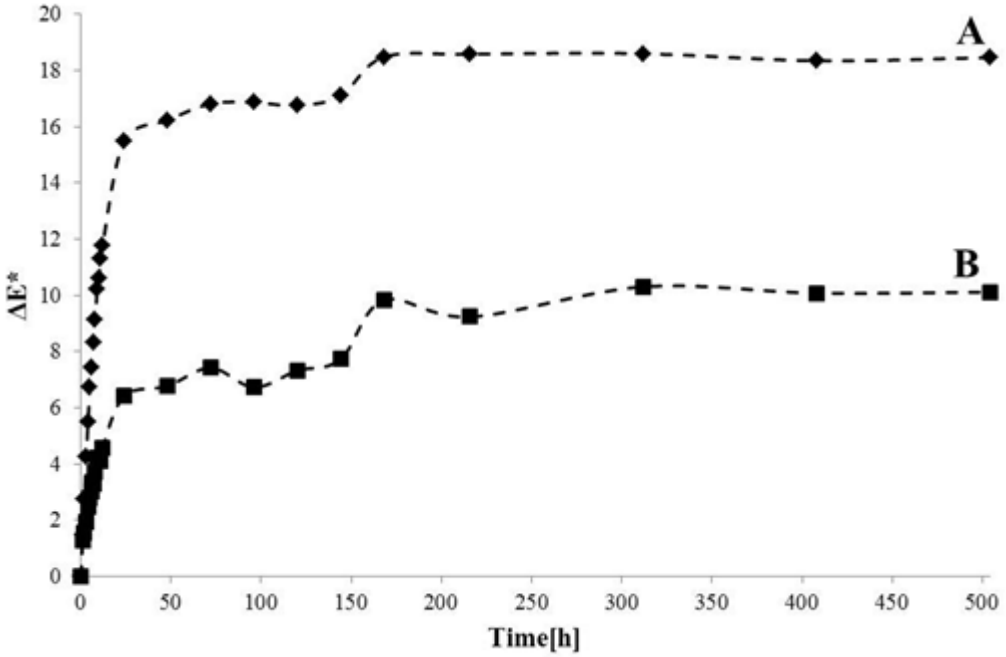


Figure 2. Changes in ΔE* values due to exposure in Solar Box of the normal (A) and red heart wood (B) over 504h.

By comparing the values of DE* in normal and red heartwood, it can be stressed that in both cases an increasing trend has been observed and that the greatest variation occur within the first 24-48 h as observed by other authors [26,43]. In both cases, after 168 h of exposure, a plateau has been reached in the DE* values, even if normal wood undergoes greater total color changes in comparison with red heartwood, as shown in Figure 2.

Multivariate significance test (MANOVA) was applies to the colorimetric measurements and significant differences among the types (normal and red heartwood; p-level < 0.001), among all the exposure times (0e504 h; p-level < 0.001) and among the time VS type (p-level < 0.001) were checked. The results of the inference statistics demonstrate that the difference between normal and red heartwood with respect to the time is highly significant even if the experimental data showed that the chromatic coordinates seemed to converge towards similar values.
The statistical regression analysis was applied to the relationships between the chromatic coordinates and the exposure times. The overall results are shown in Table 2. These regressions (Table 2) show the polynomial equations for every variable L*, a* and b* as function of the exposure times (Figure 3).
All constants of the polynomials are characterized by highly statistical significance ($p < 0.01$).
As can be observed in Figure g. 3, wood color varies with the exposure time, in particular, lightness (L*) values decrease for normal wood (darkening) and increase for red heartwood (lightening).
The same trend can be observed for the coordinate a*. At last, b* coordinate increases both in normal and red heartwood (yellowing).
At last, for normal and red heartwood a regression analysis was applied to the time in function of color coordinates (Table 3). The results shown in Table 3 underline the highly statistical significance of the obtained measures regarding the dependent variable time (t) as function of the color coordinates (L*, a* and b*).

Table 2. Regression analysis for the dependent variables L*a*b* of normal wood and red heartwood as function of the time (t).

	Normal wood		**Red heartwood**	
	Variable coefficient	**p-level**	**Variable coefficient**	**p-level**
	L*		**L***	
Intercept	76.615	<0.01	64.515	<0.01
t	-0.045	<0.01	0.021	<0.01
t^2	0.00005	<0.01	-0.00002	<0.01
	R^2adj.=0.541; p<0.01 L*= 76.615-0.045t+0.00005t^2		R^2adj.=0.495; p<0.01 L*= 64.515+0.021t-0.000002t^2	
	a*		**a***	
Intercept	6.963	<0.01	10.067	<0.01
t	0.020	<0.01	-0.008	<0.01
t^2	-0.00002	<0.01	0.00001	<0.01
	R^2adj.=0.489; p<0.01 a*=6.963+0.020-0.00002t^2		R^2adj.=0.467; p<0.01 a*=10.067-0.008t+0.00001t^2	
	b*		**b***	
Intercept	22.19	<0.01	22.954	<0.01
t	0.053	<0.01	0.057	<0.01
t^2	-0.00006	<0.01	-0.00006	<0.01
	R^2adj.=0.684; p<0.01 b*=22.19+0.053t-0.00006t^2		R^2adj.=0.848; p<0.01 b*=22.954+0.057t-0.00006t^2	

Table 3: Regression analysis for the dependent variable t (time) as function of the L*a*b* coordinates, in normal wood and red heartwood.

	Variable coefficient	**p-level**
	Normal wood (data n 2160)	
Intercept	10525.09	p<0.01
L*	-256.27	p<0.01
a*	121.18	p<0.01
b*	-111.61	p<0.01
L*^{2}	1.67	p<0.01
a*^{2}	-11.27	p<0.01
b*^{2}	3.03	p<0.01
	R^2 adj.=0.891; p<0.01 t=10525.09-256.27L*+121.18a*-111.61b*+1.67L*^{2}-11.27*^{2} +3.03b*^{2}	
	Red heartwood (data n 2160)	
Intercept	9925.968	p<0.01
L*	-137.215	p<0.01
a*	-509.234	p<0.01
b*	-218.429	p<0.01
L*^{2}	1.004	p<0.01
a*^{2}	24.137	p<0.01
b*^{2}	4.599	p<0.01
	R^2 adj.=0.889; p<0.01 t=9925.968–137.215L*-509.234*-218.429+1.004L*^{2}+24.137*^{2}+4.599b*^{2}	

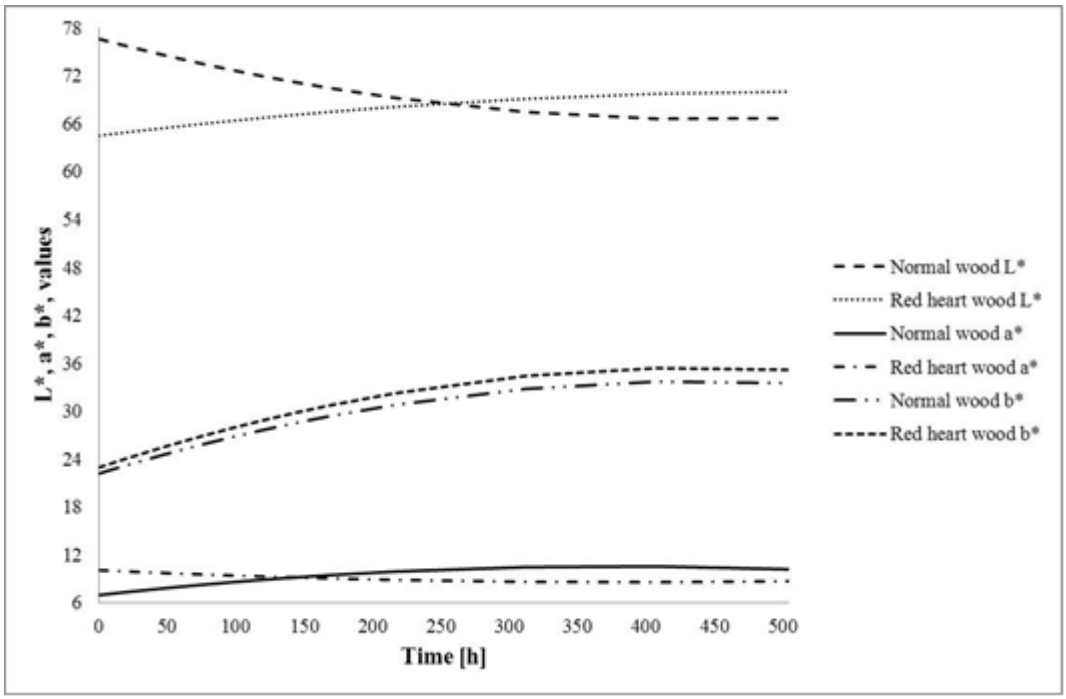


Figure 3. Colour changes of normal and read heart wood due to irradiation. L*, a* and parameters at different exposure times as calculated from the regression analysis shown in Table 2.

3.2. FT-IR analysis on the wood slices

FT-IR spectra were collected in DRIFT modality directly on wood surface in order to avoid analyzing also the unaltered wood [50,51] (Figures 4, 5). Band assignment was made according to literature references [33,52-54] (see Table 4). By observing the irradiating-time dependent diffuse reflectance FT-IR spectra of normal and red heartwood are (Figures 4, 5), it can be derived that the intensity of the bands at 1507 cm^{-1},1594 cm^{-1} and 1465 cm^{-1}, associated to lignin, decreases during photodegradation.

This is accompanied by an increase in the intensity of the band at 1739 cm^{-1}, due to carbonyl absorption. The intensity of the peak associated to carbohydrates at 1375 cm^{-1} is not significantly affected by irradiation, so this band has been used as internal reference to evaluate the lignin decay [24,25,29,21,55].

In order to determine the rate of lignin decay and carbonyl formation, the intensities of the lignin band at 1507 cm^{-1}, the carbonyl band at 1739 cm^{-1} and the carbohydrate reference band at 1375 cm^{-1} were calculated [21]. Then the relative change in ratio of lignin/carbohydrate bands at different exposure times were derived and statistically evaluated.

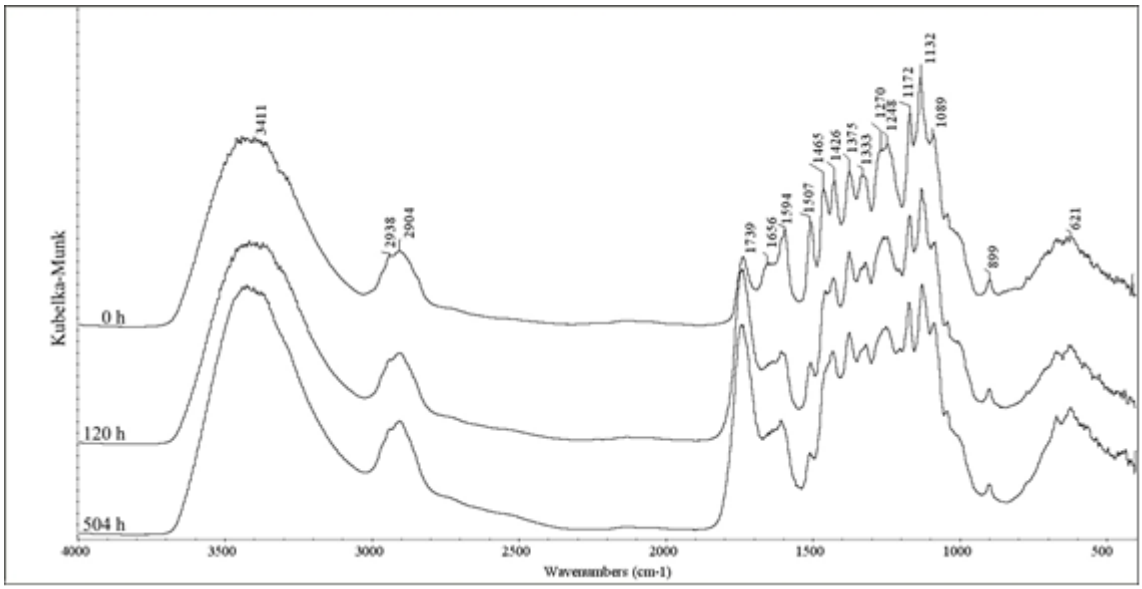


Figure 4. Diffuse reflectance spectra of normal wood slices measured at chosen exposure times

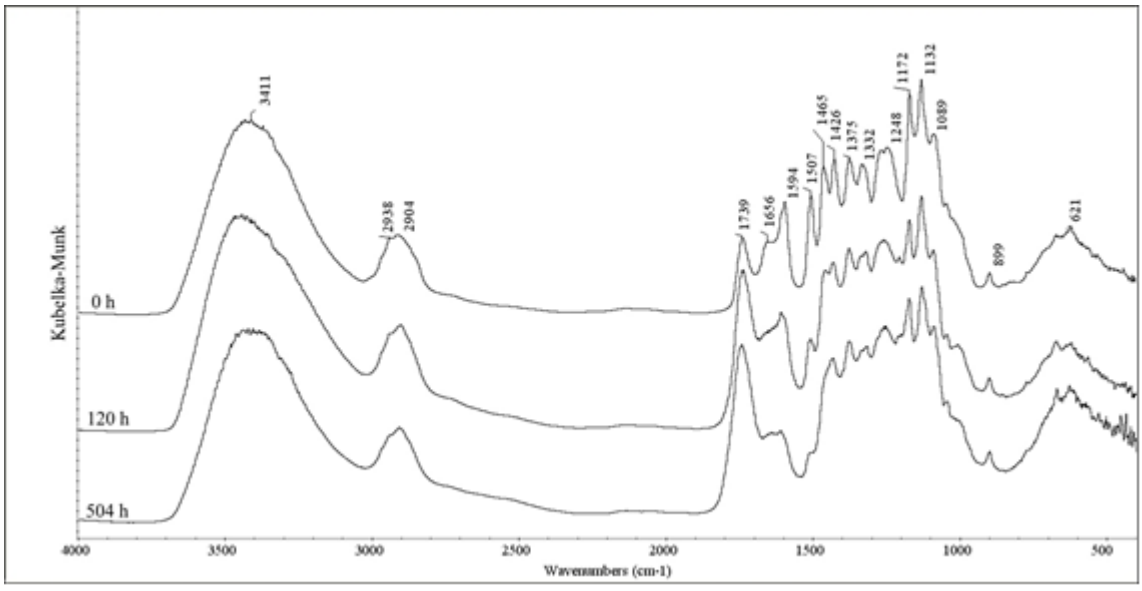


Figure 5. Diffuse reflectance spectra of red heartwood slices measured at chosen exposure times.

Table 4 Position and assignment of the bands of DRIFT spectra.

Band position (cm^{-1})	Assignment
3411	stretching of O-H group
2938	C-H and CH_2 asymmetric stretching
2904	C-H and CH_2 symmetric stretching
1739	stretching of the carbonyl group C=O
1656	conjugated carbonyl
1594	aromatic skeletal vibrations
1507	aromatic skeletal vibrations
1465	C-H deformation and aromatic skeletal vibrationsn
1426	C-H in-plane deformation
1375	C-H in-plane deformation for polysaccharides
1332	syringyl ring breathing and C-O stretching
1270-1248	guaiacyl ring breathing and C-O stretching
1172	C-O-C antisymmetric bridge stretching vibration in cellulose and hemicelluloses
1132	C-O-C symmetric stretching, aromatic C-H in-plane deformation, glucose ring vibration
1089	C-O and O-H association bands in cellulose and hemicelluloses
899	C_1-H deformation of cellulose
621	C-OH out-of-plane bending in cellulose

Table 5. T-test for dependent samples on normal and red heart wood.

	Average	**Standard deviation**	**N**	**Diff.**	**Standard deviation**	**T**	**Degree of freedom**	**p-level**
I_{1507}/I_{1375} Normal	0.855713	0.475166	14	-0.04369	0.244124	-0.66961	13	>0.05
I_{1507}/I_{1375} Red heart	0.812024	0.511930						
I_{1739}/I_{1375} Normal	3.161474	0.960797	14	-0.07445	0.734234	-0.37942	13	>0.05
I_{1739}/I_{1375} Red heart	3.087020	0.751920						

The inference statistics was applied to the I1507/I1375 and I1739/I1375 peak ratios as function of the time. In particular, the T-test for dependent samples was applied to the peak ratios as function of the time in order to verify if statistically significant differences can be found between normal and red heartwood. The results shown in Table 5 demonstrate that no statistically significant differences exist between the two types of wood, in fact the p values are always >0.05. This outcome points out that the phenolic extractives [17,56,57], that modify the beech wood color affected by red heart, do not protect this wood from photo-oxidation, as it seems to occur in the heartwood of other differentiated species such as chestnut [29] and oak [47-49,58].

According to this result, the experimental data were statistically examined without distinction between normal wood and red heartwood. The statistical regression analysis was applied to the FT-IR peak ratios and the exposure times in order to examine the relationship between them. The results

are shown in Table 6. The equations in Table 6 for the variables I1507/I1375 and I1739/I1375 as function of the exposure times, giving the second-degree polynomial curves (for I1507/I1375) and the line (for I1739/I1375), are shown in Figure 6. All constants of the polynomials and of the straight line are characterized by highly statistical significance ($p < 0.01$) (Table 6).
As can be observed in Figure 6, the FT-IR peak ratios vary with the exposure time, in particular, I1507/I1375 decrease and I1739/I1375 increase.
At last the color changes of wood during irradiation were correlated with lignin decay and the formation of carbonyl groups produced by the photo-degradation process [26]. In fact, as the color changes of the surfaces are mainly due to the formation of C=O groups due to photo-degradation of lignin, a correlation between the color coordinates and the relative intensity of the infrared peaks associated to lignin has been verified.

Table 6. Regression analysis for the dependent variables I_{1507}/I_{1375} and I_{1739}/I_{1375} as function of exposure time (t).

	Variable coefficient	**p-level**
	I_{1507}/I_{1375}	
Intercept	1.431	<0.01
t	-0.006	<0.01
t^2	0.000007	<0.01
	R^2adj.=0.777; p<0.01	
	$I_{1507}/I_{1375} = 1.431-0.006t+0.000007t^2$	
	I_{1739}/I_{1375}	
Intercept	1.45	<0.01
T	0.004	<0.01
	R^2adj.=0.412; p<0.01	
	$I_{1507}/I_{1375} = 1.45+0.004t$	

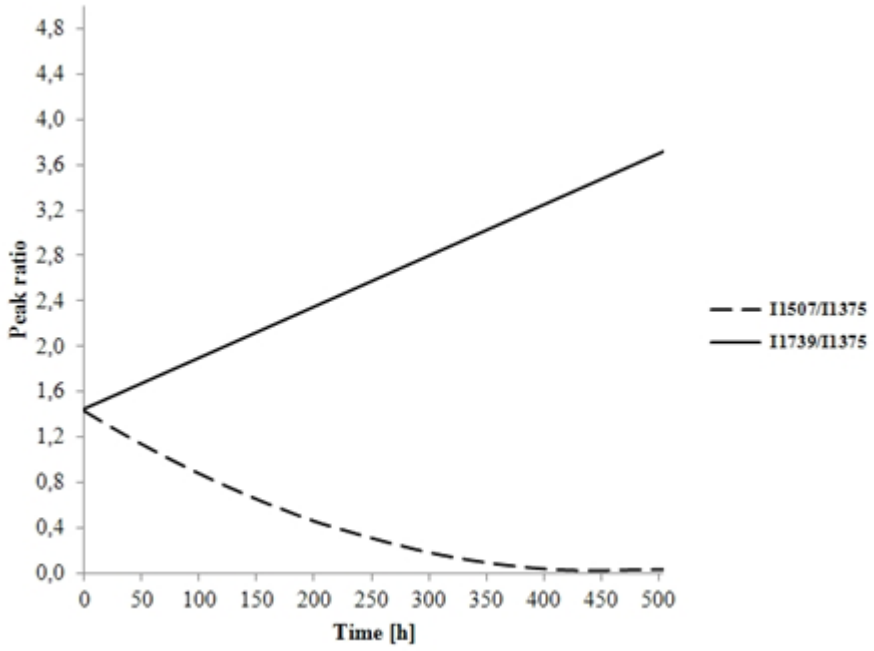


Figure 6. Change in the ratio band at different exposure times, as calculated from the regression analysis shown in Table 6.

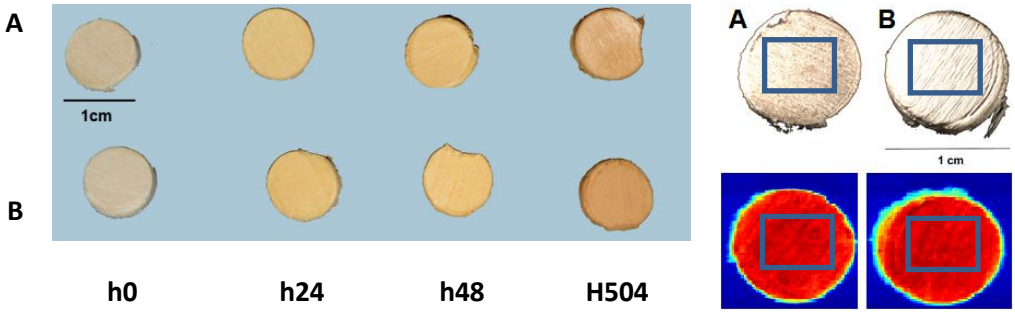
The results of the regression analysis highlight that only the I1507/I1375 peak ratio has a statically significant relation with the chromatic coordinates, in particular with L* and a* (see Table 7). The polynomial function derived by the application of the regression analysis exhibits high statistical significance ($p < 0.01$). Only the chromatic coordinates L* and a*, that represents the lightness and the green-red axes, are influent in relation to the peak ratio suggesting that they can be used to evaluate the photodegradation of wood. This result is in accordance with Müller et al. who reported a correlation between the changes of the IR band at 1510 cm_1 and DE, suggesting that the lignin decay and the resulting quinone formation is related to the photoyellowing [59].

Table 7 Regression analysis for the dependent variable I_{1507}/I_{1375} as function of the chromatic coordinates

	Variable coefficient	**p-level**
Intercept	-21.948	<0.01
a*	2.95	<0.01
L*²	0.002	<0.01
a*²	-0.163	<0.01
	R^2adj.=0.892; p<0.01 I_{1507}/I_{1375} = -21.948+2.953a*+0.002L*²-	

3.3. Hyperspectral imaging on the wood slices

The acquired beech wood samples at different irradiated times (0, 24, 48 and 504 h) are shown in Figure 7a. Spectra have been collected starting from selected ROIs (Regions of Interest) on the wood sample surfaces (Figure 7b).



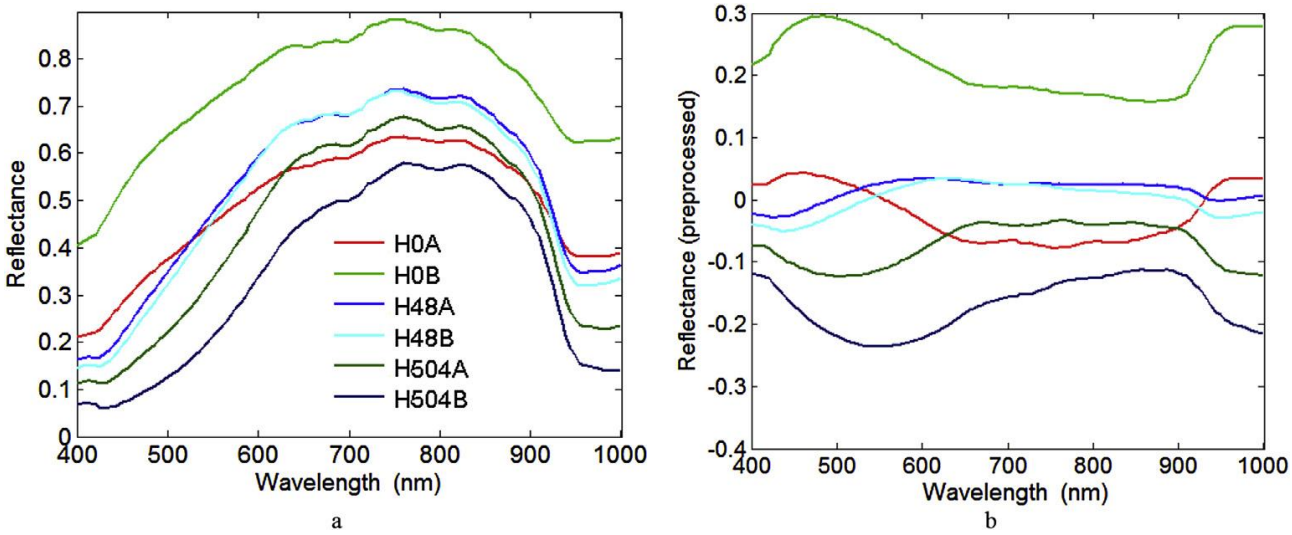
6a 6b

Figure 7. a: Images of beech wood samples analysed by HSI in the VIS-NIR and SWIR range. A: red heart wood; B: normal wood. b: Example of ROI selection on the RGB images (top) and on the corresponding false colour image related to the acquired hypercube (bottom). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3.1. VIS-NIR wavelength range

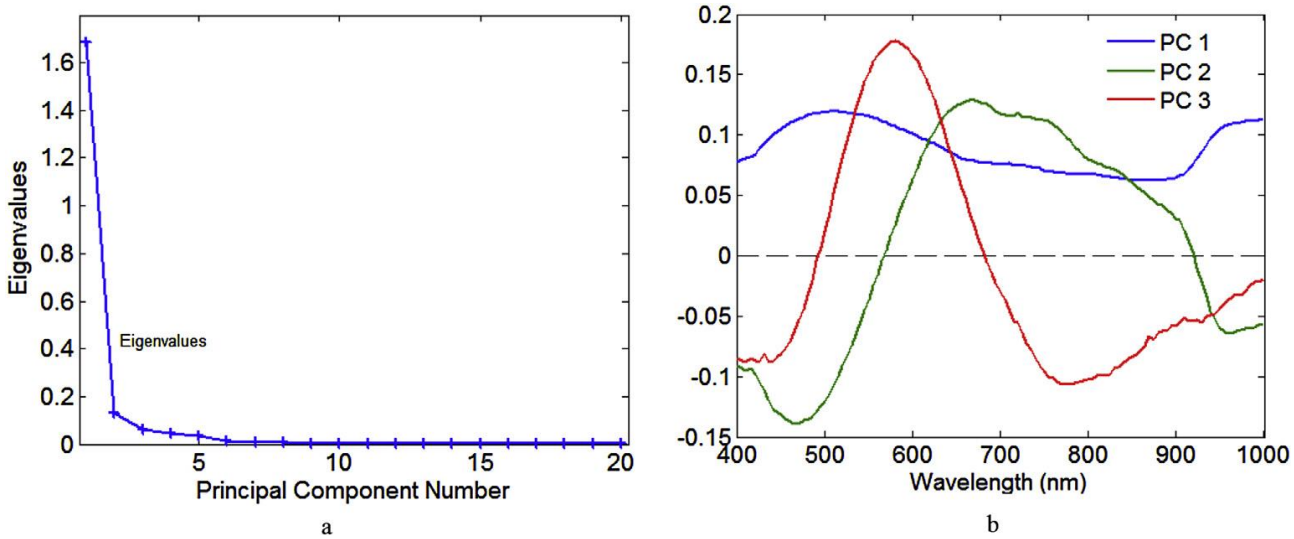
The source and preprocessed reflectance spectra related to the different exposure times of the investigated beech wood samples are reported in Figure 8. The source spectra (Figure 8a) are characterized by a quite similar shape, but different reflectance levels, the latter decreasing from H0 to H504, for both A and B wood typologies (Figure 8a).

More in details, it can be noticed that the spectrum of red heartwood at time zero is characterized by a lower reflectance compared to the spectrum of normal wood at the same time.
Concerning the spectral changes as function of irradiation time, reflectance decreases markedly in normal wood, varying from 0.4 at time 0 to 0.1 after 504 h of exposure, in agreement with what observed for other wood species [24]. The greatest change occurs during the first 24 h of exposure, in fact the spectral difference between red heartwood and normal beech wood is reduced at time 24 h. Such result is in agreement with what observed by the colorimetric measurements. The changes in spectra shape during the irradiation time and the decrease of reflectance levels in both wood sample typologies clearly indicate a darkening of the wood surface.
The application of mean centering (Figure 8b) was carried out to focus the attention on the variations between individual wood samples rather than on the absolute reflectance level. Mean centering consists simply in a subtraction of the average reflectance at each wavelength (mean level), so that the reflectance at each wavelength adds up to zero across all samples [60].
In Figure 9 the Eigenvalues of the principal components (PCs) and the loadings of the selected PCs are reported. From the graph of the Eigenvalue/principal component (Figure 9a), it is possible to observethat the greater part of the variance was captured by the first 3 principal components (PC1, PC2 and PC3), that were therefore selected. Loadings (Figure 9b) of PC1 explain variance caused by different reflectance at different aging times, loadings of PC2 andPC3 explai n the change of color towards the red.
In Figure 10 three different PCA score plots are reported: PC1 vs. PC2 for red heartwood (Figure 10a), PC1 vs. PC2 for normal wood (Figure 10b) and a 3D score plot of the first 3 PCs for both wood typologies (Figure 10c). PC1, PC2 and PC3 explained 82.25, 6.24% and 3.08% of the variance, respectively. The spectral data of the six wood samples are clustered into distinct groups according to their spectral signatures, with the exception of the red heart and normal samples aged 24 h. An evolution trend based on the increase of the exposure time is observed for both normal and red heartwood. For normal wood a change can be noticed from positive to negative values of PC1, for red heartwood there is a change first from negative to positive values of PC2 (A0 and A24) and then towards negative values of PC1 (A24 and A504). In fact, sample A0 shows more negative values of PC2 in comparison to the other samples, but values of PC1 similar to those of samples aged 24 h, probably due to its reddish color.



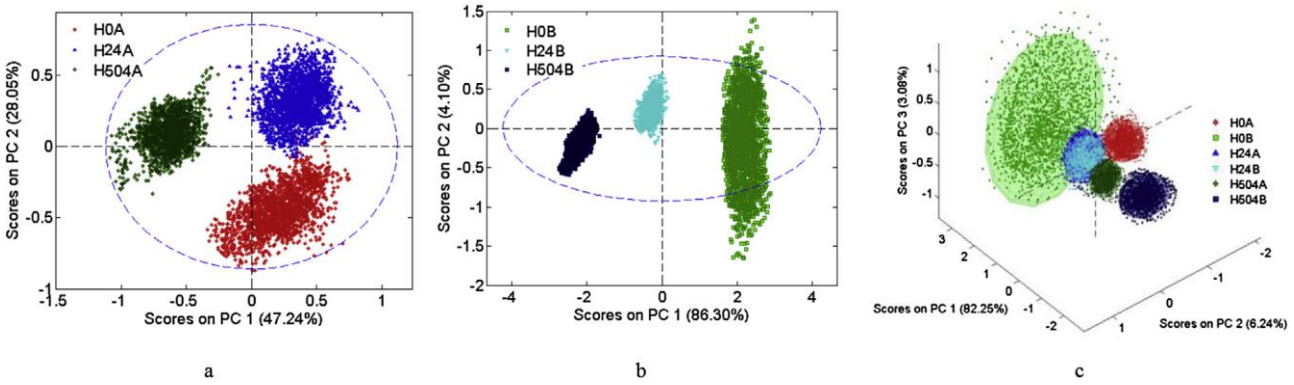
a b

Figure 8. a: Average reflectance spectra of red heart (A) and normal (B) wood characterized by 3 different exposure times (H0, H24 and H504) acquired in VIS-NIR range. b: Corresponding preprocessed spectra after the application of mean centering.



a b

Figure 9. a: Eigenvalues for all PCs. b: Loadings of the selected PCs.



a b c

Figure 10. a: PCA score plot (PC1 vs. PC2) of red heartwood (A); b: PCA score plot (PC1 vs. PC2) of normal wood (B); c: PCA 3D score plot (PC1, PC2 and PC3) of both wood sample typologies in the VIS-NIR range.

3.3.2. SWIR wavelength range

The average source and preprocessed reflectance spectra of red heart and normal wood acquired in the SWIR at different aging times are reported in Figure 11. Looking at the source spectra (Figure 11a), the strong absorptions at around 1460 nm and 1930 nm can be attributed to the combination modalities of OH in water molecules. It should be noted that the water chemical bond vibration around 1460 nm may overlap with those of other OH groups in cellulose molecules or of CH_2 groups in lignin [40], making difficult the accurate band assignment in this region. The weak spectral feature around 1730 nm may be attributed to the presence of OH bond of water molecule in cellulose/lignin and the weak absorption at 1790 nm to O-H stretching in water molecules. The mean absorptions in the 2000-2500 nm wavelength interval can be attributed to the different configurations of the C-H, C=O, C=C, and -COOH functional groups in cellulose and lignin [61]. The spectral signature of red heartwood and normal wood at time 0 is very similar.

Concerning preprocessing, 1st Derivative was applied for removing offset difference and SNV for removing the constant effect of scattering (Figure 11b).

In Figure 12 the Eigenvalues of the principal components (PCs) and the loadings of the selected PCs are reported. From the graph of the Eigenvalue/principal component (Figure 12a), it is possible to observe that the greater part of the variance was captured by the first 3 principal components (PC1, PC2 and PC3), that were therefore selected. Loadings (Figure 12b) of PC1 explain variance caused by absorption at 1400 and 1900 nm related to hygroscopic water, PC2 and PC3 explain the chemical variation of cellulose/lignin absorption during the aging time.

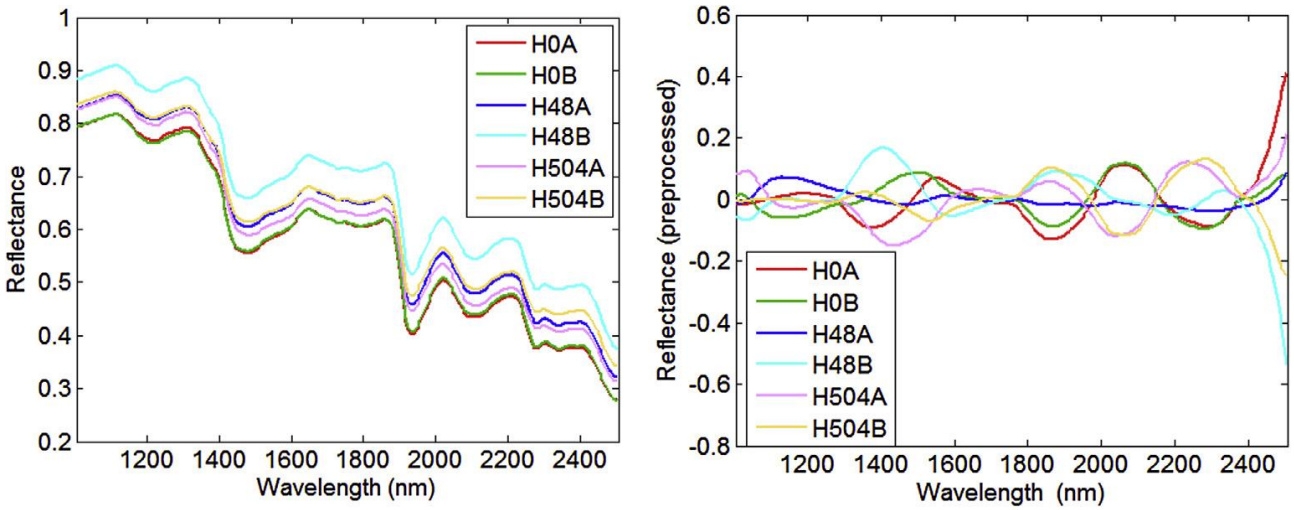


Figure 11. a: Average reflectance spectra of red heart (A) and normal (B) wood characterized by 3 different exposure times (H0, H48 and H504) acquired in the SWIR range. b: Corresponding preprocessed spectra after the application of 1st Derivative, SNV and mean center. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

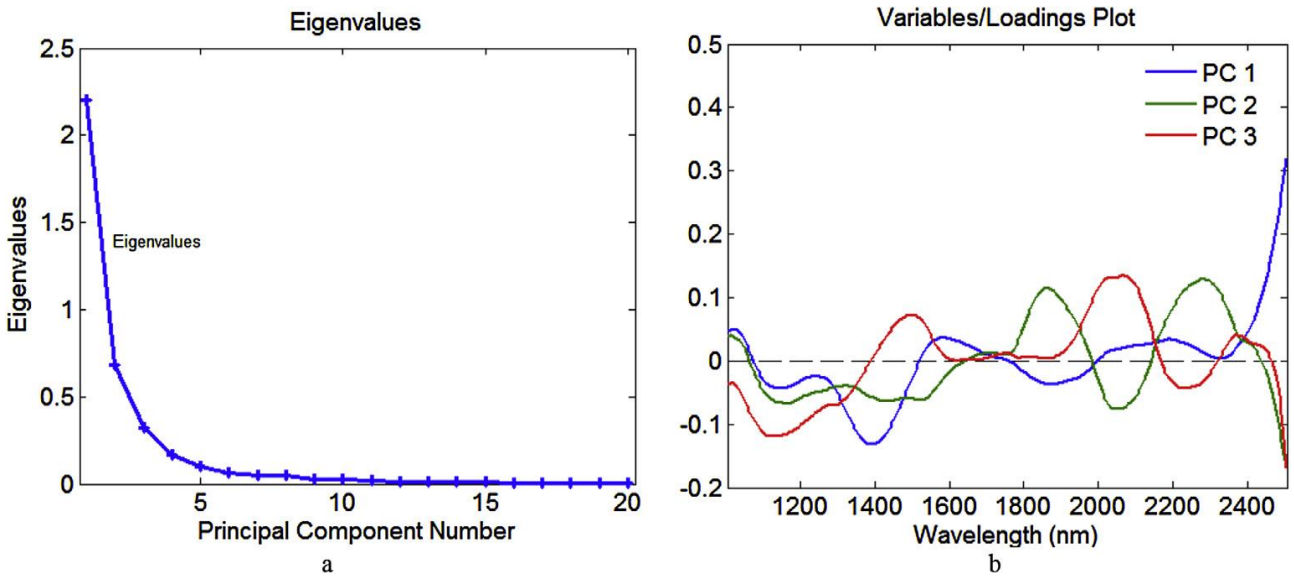
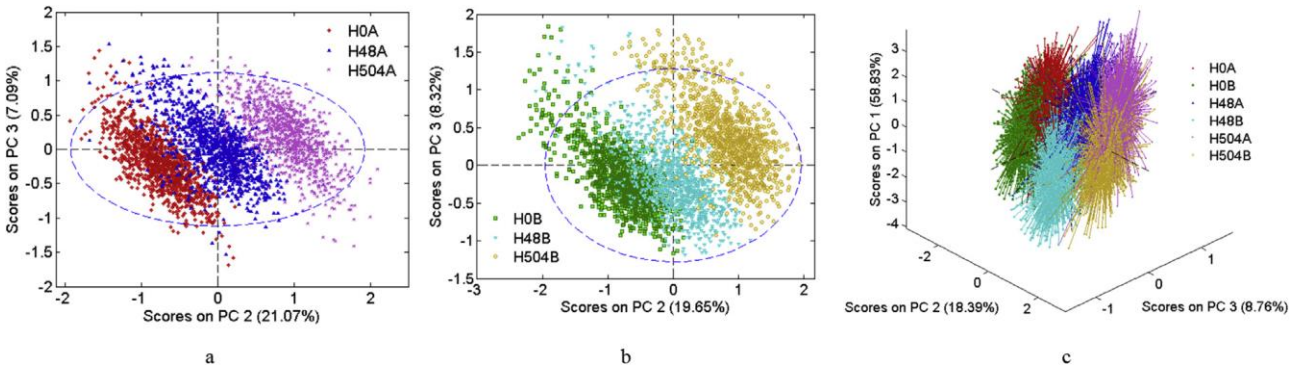


Figure 12. a: Eigenvalues for all PCs. b: Loadings of the selected PCs.

In Figure 13 three different PCA score plots are reported: PC2 vs. PC3 for red heartwood (Figure 13a), PC2 vs. PC3 for normal wood (Figure 13b) and a 3D score plot of the first 3 PCs for both wood typologies (Figure 13c). PC1, PC2 and PC3 explained 58.63, 18.39 and 8.76% of the variance, respectively. Comparing the samples of red heartwood and normal wood it appears that for every aging time they are clustered in the same regions of the score plot, indicating that no significant differences are detected. Moreover, comparing the different aging time, also in this case it can be noticed an evolution trend of both wood sample typologies, according to the increase of irradiation time. Such trend is evident looking at the values of PC2, varying from negative to positive values for samples aged from time 0 to 504 h. The biggest variation occurred after 48 h, in agreement with what observed by FT-IR spectroscopy. The observed changes are probably related to the decrease of cellulose and lignin due to photodegradation.



a b c

Fig. 13. a: PCA score plot (PC2 vs. PC3) of red heartwood (A); b: PCA score plot (PC2 vs. PC3) of normal wood (B); c: PCA 3D score plot (PC1, PC2 and PC3) in the SWIR range. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

Color changes in normal and red heartwood due photoirradiation have been investigated applying different methods. The reflectance spectrophotometry pointed out that the greatest changes occur in normal wood within the first hours of exposure and they are mainly due to L* decrease and b* increase. Color modifications observed in normal and red heartwood tend to converge toward similar chromatic coordinates even if the differences before the irradiation are clearly visible with the naked eyes.

The results of the inference statistics indicated also that the difference between normal and red hearted wood with respect to the time is highly significant even if the experimental data showed that the chromatic coordinates seemed to converge towards similar values.

The regression analysis underlines the highly statistical significance of the obtained measures regarding the dependent variable time as function of the color coordinates, in both red heart and normal wood.

FT-IR spectroscopy highlighted the rate of photo-degradation of wood surface due to lignin oxidation, by studying the lignin/carbohydrate intensity ratio as function of time. The results revealed that lignin degrades quickly: after 48 h of irradiation the lignin/carbohydrate ratio (I1507/I1375) decreased to about 50% of its original value both in normal and red heartwood.

The T-test for dependent samples, applied to the peak ratios as function of the time, demonstrated that no statistically significant differences exist between the two types of wood: as a consequence the extractives in red heartwood do not act as antioxidants like in other species.

The regression analysis showed that the differences between the values at the various irradiation times are statistically significant.

The color changes of wood during irradiation were correlated with lignin decay and the formation of carbonyl groups produced by the photo-degradation process. The results of the regression analysis indicated that I1507/I1375 peak ratio has a statically significant relation with the chromatic coordinates, in particular with L* and a*.

Concerning hyperspectral imaging, the results obtained in the VIS-NIR range indicated that the wood samples are characterized by different spectral signatures, according to their color variations. Reflectance levels decrease increasing the irradiation time. The greatest change occurred after 24 h, in agreement with the previously reported results based on colorimetric measurements. In the SWIR range, the results showed that red heartwood and normal wood are characterized by a similar spectral signature at time 0, indicating that probably no major compositional differences are present between the two kinds of wood. Also in this wavelength range an evolution trend was observed related to the irradiation time of the wood samples, with the greatest changes after 48 h. Such results are in agreement with the results obtained by FT-IR spectroscopy, and are probably related to the alteration of lignin.

In conclusion, color measurements can be assumed as a valid and useful tool for the evaluation of wood surface modifications caused by UV irradiation, suggesting the possibility to choose this non-invasive method as a standard control test to monitor the state of preservation of the wood surfaces.

Acknowledgments
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