

Light induced colour changes and chemical modification of treated and untreated chestnut wood surface

Luca Calienno¹, Claudia Pelosi², Rodolfo Picchio¹, Giorgia Agresti², Ulderico Santamaria², Federica Balletti¹, Angela Lo Monaco¹

¹Department of Agriculture, Forests, Nature and Energy (DAFNE), University of Tuscia, Via San Camillo de Lellis, 01100 Viterbo (Italy)

²Department of Cultural Heritage Sciences (DISBEC), University of Tuscia, Largo dell'Università, 01100 Viterbo (Italy).

Corresponding author: Claudia Pelosi, pelosi@unitus.it

Abstract

The aim of this work is to study the surface modifications of chestnut (*Castanea sativa* Mill.) wood by reflectance spectrophotometry and Fourier Transform Infrared spectroscopy in order to understand the mechanisms that cause the changes and to suggest possible solutions to avoid the degradation phenomena. The experimental data were statistically treated to evaluate their significance. Concerning the surface protection of wood, starting from the results obtained by testing different commercial products, the attention has been focused on a novel organic preservative/consolidant product that seems to attract a great interest in the field of conservation of wooden artefacts.

Colour monitoring allowed to find that wood surface colour undergoes an important variation due to photo-irradiation, occurring within the first 24 hours and mainly due to L* decrease and b* increase. Though the protective treatment modifies wood colour, nevertheless the product tested seems to protect wood surface by reducing the **photoyellowish**.

FTIR analysis put in evidence that irradiation caused the degradation of lignin and increased the concentration of the chromophore groups on the wood surface. Changes in the chromatic coordinates can be linked to the degradation of lignin and to the increase of the concentration of carbonyl groups.

Keywords: wood, conservation, photo-degradation, Fourier Transform Infrared spectroscopy, reflectance spectrophotometry.

Introduction [heading]

Wood, a composite of cellulose, hemicellulose, lignin, and extractives, is commonly used as engineering and structural material. The aesthetic beauty of wood is obvious, as seen in its extensive use for veneers, floorings, frames and furniture. In cultural heritage wood has been widely used since ancient times, both as a structural building component and as a material for panels, statues, furniture (Agresti, *et al.*, 2010). As a consequence, the study of wood surface variations can be interesting both to understand the mechanisms that cause the changes and to suggest possible solutions to avoid the degradation phenomena. **In fact, unprotected wood exposed to outdoor conditions undergoes a variety of degradation reactions induced by diverse factors such as light, moisture, heat, oxygen, and pollutants, causing chemical and colour changes of wood surface (Tolvaj & Faix, 1995; Pandey & Pitman, 2003; Pandey, 2005a). Surface treatments, carried out in order to protect wood objects from these factors, modify colour (Unger, *et al.*, 2001). Moreover, regarding treated surfaces, colour changes due to sunlight exposure will depend on both wood and product used for its treatment (Biscontin, *et al.*, 2009; Genco, *et al.*, 2009; Crisci, *et al.*, 2010; Genco, *et al.*, 2011; Lo Monaco, *et al.*, 2011a, 2011b). Colour change method seems to be the most sensitive to determine the extent of photo-degradation of untreated and treated wood exposed to ultraviolet and visible radiation (Sharratt, *et al.*, 2009), although other indicators such as strength loss and weight changes can also be used (Evans, 1998; Derbyshire, *et al.*, 2009). Furthermore, in cultural heritage the monitoring of wood surfaces exposed to sunlight radiations should be performed through non-destructive methods to avoid the paradox of damaging a work of art while monitoring its preservation state (Marengo, *et al.*, 2003, 2004). Starting from these general remarks, in this paper colour and chemical modifications of chestnut wood (*Castanea sativa* Mill.) due to artificial ageing were examined. The choice of chestnut was due to its wide use in Central Italy especially for the construction of doors. Moreover, chestnut wood was never studied before according to the methodology presented in this paper. Chestnut was generally employed for the outer surface of the doors due to the properties of its heartwood. In fact, chestnut heartwood contains extractives that give it a pleasant colour and a natural durability in regards to biotic and abiotic agents. A novel preservative/consolidant product, based on natural sources, was used to treat the wooden samples. This product, named Linfoil®, is attracting an increasing interest of the conservators in the treatment of wooden objects (Lo Monaco, *et al.*, 2011a, 2011b). In Viterbo, for example, the doors of *Palazzo dei Mercanti* and of *Santa Maria della Salute* are made of chestnut wood and recently they were treated with Linfoil® by a conservator based on the experimental tests made on the product and exposed in this paper (private communication of the conservator). Colour measurements were chosen as possible method to evaluate wood surface changes. Since colour changes on wood surfaces are due to photo degradation of its chemical constituents, the study of the CIELAB colour changes and changes in chemical composition due to irradiation is of practical importance both in cultural heritage and in contemporary artefacts and objects. The chemical modifications of wood constituents caused by irradiation were examined by FTIR analysis (Agresti, *et al.*, 2013; Kataoka & Kiguchi, 2001; Moore & Owen, 2001; Pandey, 2005a; Tolvaj, *et al.*, 2011). The relative changes in intensities of spectral bands associated with lignin and carbohydrates as a result of deterioration were determined at different exposure times.**

In this work the authors would like to test a simple method to rapidly check the performance of a commercial product on chestnut wood to support the work of the conservator on real case studies.

Experimental [heading]

Sample preparation [sub-heading]

Flat wood samples (**23cm length, 5cm width and 2cm thickness**) were obtained by a board of chestnut, cut in the heartwood. After cutting, the samples were stored in darkness in a conditioned room at 65% relative humidity and a temperature of 22°C to reach the 12% of moisture content (Lo Monaco, *et al.*, 2011c). **The radial surface was used for the measurements.**

Half of the samples were treated with a novel commercial product named Linfoil®, a mixture of vegetable oils, resins and waxes in aliphatic solvent, produced by GEAL (Agliana, Pistoia, Italy). Linfoil® was diluted with Linfosolv® (1:1 volume ratio), a solvent system made of a mixture of refined paraffinic aliphatic derivatives and extracts from citrus; then three coatings of the solution were applied on the wood samples by brush. Linfoil® was previously characterized and tested for ageing (Pelosi, *et al.*, 2013).

Artificial ageing [sub-heading]

The accelerated ageing of the samples was performed in a Model 1500E Solar Box (Erichsen Instruments). The system is equipped with a 2.5 kW xenon-arc lamp and an UV filter that cuts off the spectrum at 280 nm. The samples were exposed in the Solar Box chamber from 0 to 504 h at 550 W/m², 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 light outdoor 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.**

Colour monitoring [sub-heading]

After exposure for a given length of time the samples were removed from the Solar Box chamber and the colour was measured using an X-Rite CA22 reflectance spectrophotometer, generally employed in wood technology and cultural heritage studies to evaluate the colour changes after conservation and treatment operations (Crisci, *et al.*, 2010; Genco, *et al.*, 2011; Ferreira, *et al.*, 2012). The characteristics of the colour measuring instrument are the following: colour scale CIEL*a*b*; illuminant D65; standard observer 10°; geometry of measurement 45°/0°; spectral range 400-700 nm; spectral resolution 10 nm; measurement diameter 4 mm; white reference supplied with the instrument. The CIELAB colour system was used where L* describes the lightness while a* and b* describe the chromatic coordinates on the green-red and blue-yellow axes, respectively. The differences in lightness (ΔL^*), chromatic coordinates (Δa^* and Δb^*), and total colour (ΔE^*) were then calculated using these parameters according to Normal 14/93 (1993) and EN 15886 (2010). The total colour difference, ΔE^* , between two measurements ($L^*_1 a^*_1 b^*_1$ and $L^*_2 a^*_2 b^*_2$) is the geometrical distance between their positions in CIELAB colour space. It is calculated using the following equation: $\Delta E^*_{2,1} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Measurements were taken at the following 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 hours.

To guarantee that successive measurements were made in the same area a graduated mask was used.

Concerning wood samples, one hundred and eighty colour measuring points were chosen: ninety for the untreated and ninety for Linfoil® treated wood samples. Three measures for each point were performed, according to Normal 14/83, so that five hundred and forty measurements were realized at each exposure time, then average values and standard deviations were calculated.

Fourier Transform Infrared Spectroscopy (FTIR) [sub-heading]

Infrared spectra were obtained using a Nicolet Avatar 360 Fourier transform spectrometer. For each sample 128 scans were recorded in the 4000 to 400 cm^{-1} spectral range in diffuse reflection modality (DRIFT) with a resolution of 4 cm^{-1} . Spectral data were collected with OMNIC 8.0 (Thermo Electron Corporation) software.

Slices with a size of 10 mm (diameter) x 2 mm (thickness) were used. Spectra were measured in DRIFT mode. The diameter of the wood slices was suitable for the FTIR diffuse reflectance accessory unit. As background the spectrum of the KBr powder was used.

Peak heights were measured using OMNIC software according to the method described in the literature (Pandey & Pitman, 2003).

Statistical analysis [sub-heading]

Data were analyzed with the Statistica 2010 advanced statistics software. 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 tests were conducted with Tukey HSD test method.

In the regression analysis for the colour coordinates as function of the exposure times, the two sets (untreated and treated) were considered and calculated as statistical dummy variables to develop prediction models (Picchio, *et al.*, 2012).

Results and discussion [heading]

Colour changes of wood samples [sub-heading]

Firstly it must be underlined that wood colour shows high variability, in spite of the samples were obtained by a single board to avoid the material variability, as observed in previously published papers (Lo Monaco, *et al.*, 2011a, 2011b). For this reason, it was necessary to perform a lot of measurements, 540 at each exposure time 270 for the treated and 270 for the untreated samples, in order to quantify and account for this variability and to statistically validate the experimental data. M-ANOVA and Tukey tests, applied to 504 hours of irradiation, underline that L^* , a^* and b^* undergo statistically significant changes after the exposure times both for untreated and treated samples (Table 1).

The regression analysis, applied to the chromatic coordinates as function of the exposure time, showed the statistical validity for the two set of samples (untreated and treated) by considering L^* , a^* and b^* as dummy variables (Tables 2 and Figure 1).

As can be observed in the Table 1 and Figure 1, wood colour varies with the exposure time, in particular, lightness (L^*) values decrease markedly, whereas the chromaticity parameters a^* and b^* increase, as found also by other authors (Pandey, 2005a; Sharratt, *et al.*, 2009; Tolvaj, & Mitsui, 2010).

The data reported in Table 1 show that L^* value decreases from 76.7 to 62.7, the a^* coordinate increases from 6.2 to 9.4 reaching an almost constant value after 72 h of exposure and the b^* value increases much more rapidly than a^* (from 19.1 to 28.2 within the first 24 h) attaining an almost constant value after 48 h of irradiation. **To better appreciate the changes, a bar diagram of the difference values (ΔL^* , Δa^* and Δb^*) at some chosen exposure times is also shown in figure 2. Both Δa^* and Δb^* increase, correlating to increasing yellow and red colouration, with the rise in yellow (b^* coordinate) being the greatest change. Δa^* and Δb^* changes begin to reverse after 312 h towards the 504 h (Figure 2). The reversal of colour change can be attributed to the beginning of a photo-bleaching reaction, as suggested by some authors (Sharratt, *et al.*, 2009).** In general it is possible to state that the greatest change occurs within the first 24 h of exposure and around the 470 nm ($\Delta R\%=-20.3$) and 560 nm ($\Delta R\%=-20.2$) wavelengths which correspond in blue and green shades respectively, as deduced by the reflectance spectra (Figure 3). This correlates to an increase in yellow/red colouring and is in agreement with an increase of a^* and b^* coordinates. Concerning the treated wood, it has been found that Linfoil® treatment causes a darkening and a **photoyellowish** of wood surface, as demonstrated by the variations of the chromatic coordinates ($\Delta L^*=-13.97$, $\Delta a^*=3.12$, $\Delta b^*=15.41$, $\Delta E^*=21.03$). Also in the treated samples, the colour varies with the exposure time (see Table 1, **Figures 1-2**). In particular, L^* values decrease from 62.7 to 55.0 whereas the chromaticity parameters a^* and b^* increase from 9.35 to 13.26 and from 34.5 to 37.1 respectively. Nevertheless the changes are much lower than those observed for the untreated samples, especially for the b^* coordinate **whose values decrease before 72 h of exposure and increase after this time (Figure 2).**

The greatest change in the reflectance spectra occurs around the 580 nm ($\Delta R\%=-9.24$) wavelength which corresponds in green/yellow shade, as deduced by the reflectance spectra (Figure 4). Nevertheless, it must be stressed that the reflectance changes due to exposure are much lower in the treated samples with respect to untreated ones. The minor colour changes of Linfoil® treated samples could be attributed to a protective effect of this compound.

The analysis of Linfoil® by FTIR spectroscopy and analytical pyrolysis coupled to a gas chromatographic-mass spectrometric system (Pelosi, *et al.*, 2013) demonstrated that it is composed of alkyd resins and additives, and that it exhibits a high stability to the photo-degradation, in accordance with the findings by other authors for alkyd resin composite (Peng, 2012).

By analyzing the obtained results, it is possible to suppose that the rapid decrease of lightness observed for the untreated wood samples within the first 24 h of exposure, can be ascribed to photo-degradation processes. The photo-degradation is primarily related to the decomposition of lignin which leads to the generation of chromophoric groups absorbing especially in the UV range of the sunlight spectrum (Hon, 2001; George, *et al.*, 2005; Pandey, 2005a).

The rapid decrease of L^* values can be associated also to the photo-degradation of extractives, followed by a rapid oxidation of the degradation products, as discussed by Tolvaj and Mitsui (Tolvaj, & Mitsui, 2010). Moreover phenolic extractives undergo rapid photochemical change due to their good absorption of light (Pandey, 2005b). On the other hand, some authors (Zahri, *et al.*, 2007) demonstrated a good resistance to

photo-degradation of some extractives, in particular they underlined that gallic acid is much more stable than ellagic acid after UV light exposure. The degradation of extractives and the rapid oxidation of degradation products may act as protection of the main wood components (Nzokou & Kamdem, 2006; Chang, *et al.*, 2010). A similar behaviour was observed for wood samples treated with preservative products (Nzokou & Kamdem, 2006; Lo Monaco, *et al.*, 2011a, 2011b). Some authors suggested that the high durability of American chestnut could be the result of a coupling of tannin and lignin providing a “fixed preservative” where the ellagate moiety is resistant to oxidative degradation (Helm, *et al.*, 1997).

FTIR analysis on wood slices [sub-heading]

The infrared spectra of chestnut slices at chosen exposure times are shown in Figure 5. Band assignment was made according to literature references (Moore & Owen, 2001; Chang, *et al.*, 2002; Colom, *et al.*, 2003; Pandey & Pitman, 2003; Pelosi, *et al.*, 2013). FTIR spectrum shows the following bands (see Figure 4, 0h): 1740 cm^{-1} stretching of the carbonyl group C=O, 1598 cm^{-1} aromatical skeletal vibrations, 1507 cm^{-1} aromatic skeletal vibrations, 1465 cm^{-1} C-H deformation and aromatical skeletal vibrations, 1427 cm^{-1} C-H in-plane deformation, 1377 cm^{-1} C-H in-plane deformation for polysaccharides, 1340-1327 cm^{-1} syringyl ring breathing and C-O stretching, 1250 cm^{-1} guaiacyl ring breathing and C-O stretching, 1168 cm^{-1} C-O-C antisymmetric bridge stretching vibration in cellulose and hemicelluloses, 1131 cm^{-1} C-O-C symmetric stretching and aromatic C-H in-plane deformation and glucose ring vibration, 1082 cm^{-1} C-O and O-H association bands in cellulose and hemicelluloses, 898 cm^{-1} C₁-H deformation of cellulose.

It can be observed that the intensity of the bands at 1507 cm^{-1} , 1598 cm^{-1} and 1465 cm^{-1} , associated to lignin, decrease during photo-degradation. This is accompanied by an increase in the intensity of the band at 1740 cm^{-1} , due to carbonyl absorption. The intensities of the peak associated to carbohydrates at 1377 cm^{-1} is not significantly affected by irradiation, so this band has been used as internal reference to evaluate the lignin degradation.

In order to determine the rate of lignin degradation and carbonyl formation, the intensities of the lignin band at 1507 cm^{-1} , the carbonyl band at 1740 cm^{-1} and the carbohydrate reference band at 1377 cm^{-1} were calculated (Pandey & Pitman, 2003). The relative change in ratio of lignin/carbohydrate bands at different exposure times is showed in Figure 6. The lignin/carbohydrate intensity ratio decreased with the exposure times and after 168 h of irradiation it decreased to about 50% of its original value. The relative increase of the carbonyl groups during photo-irradiation was calculated by considering the ratio of intensity of carbonyl band at 1740 cm^{-1} against reference peak at 1377 cm^{-1} (Figure 7). The intensity of the band of the carbonyl groups increases as function of irradiation time. This can be explained by the formation of C=O groups of degraded lignin.

As the carbonyl groups increase during the photo-ageing whereas the lignin band decreases, the ratio of intensity of the bands at 1507 and 1740 cm^{-1} undergoes a fast and significant decrease after few hours of exposure, as can be observed in Figure 8. In fact, after 48 hours of exposure the experimental value of the I_{1507}/I_{1740} ratio decreased to about 40% of its original value.

Relation between colour and chemical changes in wood samples [sub-heading]

At last the colour changes of wood during irradiation were put in relation with the lignin degradation and the formation of carbonyl groups produced by the photo-degradation process. In particular, L*, a* and b* values were reported as function of infrared peak ratio in order to study the trend of the data. In fact, as the colour changes of the surfaces are mainly due to the formation of C=O groups due to photo-degradation of lignin, there could be a relationship between the chromatic coordinates and the relative intensity of the infrared peaks associated to lignin.

The results in Figures 9-11 show that the lignin degradation is linked to photo-induced colour changes of wood surfaces. In particular, L* decreases as function of the peak ratios whereas a* and b* undergo a little increase. This result suggests that the **extractive-rich** wood degradation, investigated through FTIR analysis, can be put in relation to the colour modification of the surfaces. **This result was demonstrated also for poplar wood (Agresti, et al., 2013).**

Conclusions [heading]

The study of colour changes in chestnut samples showed that wood exhibits high colour variability. The colour measurements have to be realized exactly in the same points during the time in order to avoid calculating surface variability rather than colour changes of wood due to simulated sunlight exposure. Wood surface colour undergoes an important variation due to photo-irradiation. The greatest changes occur within the first 24 hours and they are mainly due to L* decrease and b* increase. Linfoil® treatment modified wood colour. Nevertheless, wood samples treated with Linfoil® exhibited colour changes much lower than those of untreated samples. This product seems to protect wood surface by reducing the **photoyellowish**.

FTIR spectroscopy allowed investigating the rate of photo-degradation of wood surface due to lignin oxidation. The rate of photo-degradation was investigated by studying the lignin/carbohydrate intensity ratio as function of time. The results put in evidence that the I₁₅₀₇/I₁₃₇₇ ratio (lignin/carbohydrate) decreased to about 50% of its original value after 168 hours of exposure.

An important result was that the colour changes, expressed as L*, a*, b* coordinates variations, can be related to the photo-degradation of lignin obtained by FTIR analysis. This finding encourages to further explore the relation between colour changes and chemical modification of wood surface. This is of particular importance in the field of cultural heritage because allows to control the artefact surfaces after the conservation work in a simple and economic method. The role of extractives needs to be further investigated in order to better understand their influence in the surface wood changes, especially regarding outdoor artefacts made of wood containing hydro soluble tannins, like chestnut.

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Figure and Table Captions

Figure 1 Colour changes of untreated (UT) and Linfoil® treated (T) chestnut samples due to photo-irradiation. Variation of L^* , a^* and b^* parameters at different exposure times as calculated from the regression analysis shown in Table 2.

Figure 2 Changes in CIE- $L^*a^*b^*$ parameters of untreated (UT) and Linfoil® treated (T) chestnut samples, as shown by ΔL^* , Δa^* and Δb^* due to accelerated sunlight exposure, at chosen exposure times.

Figure 3 Variation in the reflectance spectra of untreated chestnut samples due to photo-irradiation. Focus on the changes occurring at specific wavelengths within 504 h.

Figure 4 Variation in the reflectance spectra of Linfoil® treated chestnut samples due to photo-irradiation. Focus on the changes occurring at specific wavelengths within 504 h.

Figure 5 Infrared spectra of chestnut slices measured at different exposure times.

Figure 6 Change in the ratio of lignin band at 1507 cm^{-1} against the carbohydrate band at 1377 cm^{-1} , as function of the irradiation time on the chestnut wood slices.

Figure 7 Change in the ratio of carbonyl band at 1740 cm^{-1} against the carbohydrate reference band at 1377 cm^{-1} , as function of the irradiation time, on the chestnut wood slices.

Figure 8 Change in the ratio of lignin band at 1507 cm^{-1} against the carbonyl band at 1740 cm^{-1} , as function of the irradiation time, on the chestnut wood slices.

Figure 9 L^* , a^* , b^* coordinates as function of the infrared peak ratio I_{1507}/I_{1377} (lignin/carbohydrate)

Figure 10 L^* , a^* , b^* coordinates as function of the infrared peak ratio I_{1740}/I_{1377} (carbonyl/carbohydrate)

Figure 11 L^* , a^* , b^* coordinates as function of the infrared peak ratio I_{1507}/I_{1740} (lignin/carbonyl)

Table 1

Averages of chromatic coordinates at different time of exposure in Solar Box for untreated (UT) and Linfoil® treated (T) chestnut wood samples. M-ANOVA test shows highly significant differences of the colorimetric parameters at different exposure times. Different letters indicate statistical differences (Tukey test, $\alpha = 0.01$).

Table 2

Regression analysis applied to the chromatic coordinates as function of the exposure times in Solar Box for untreated and Linfoil® treated chestnut samples.

List of suppliers

Linfoil® and Linfosolv® were supplied by GEAL Via Settola 121, 51031 Agliana (Pistoia), Italy. Tel (+39) 0574 750365 - info@geal-chim.it.

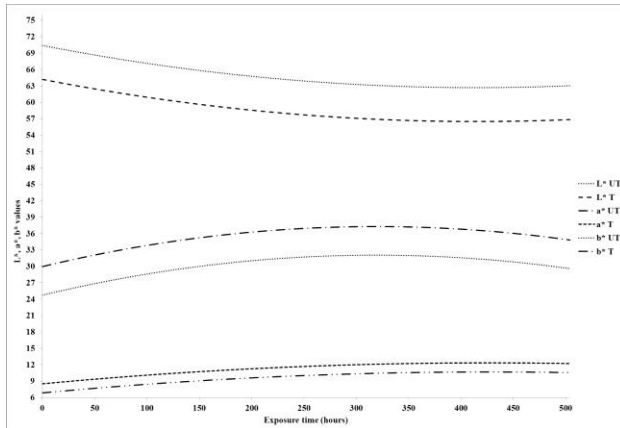


Figure 1

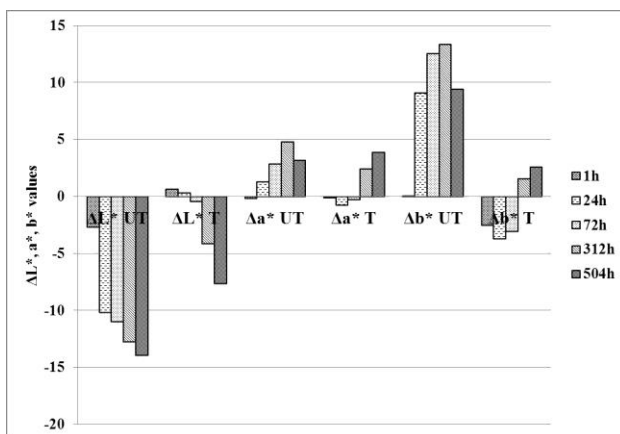


Figure 2

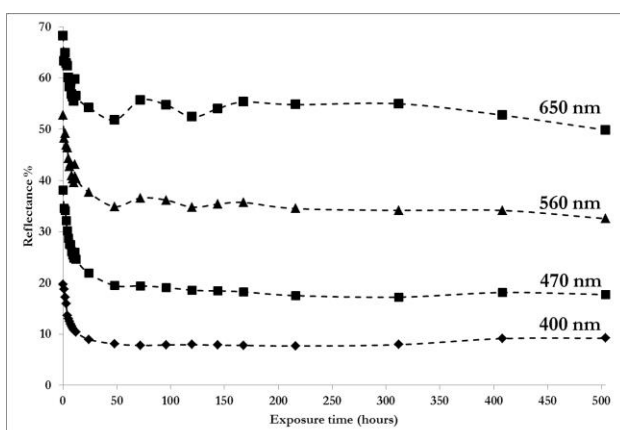


Figure 3

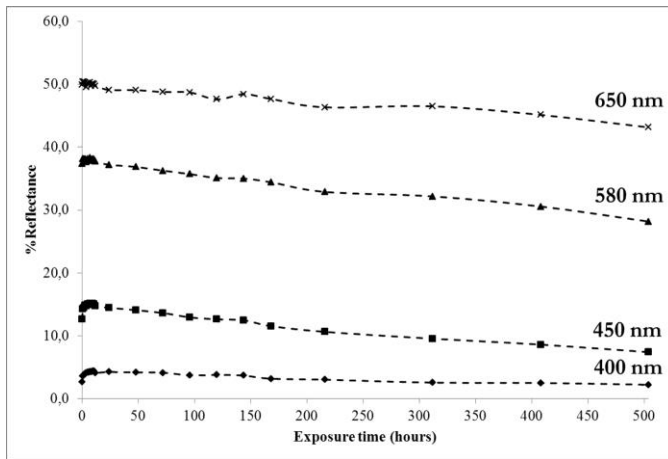


Figure 4

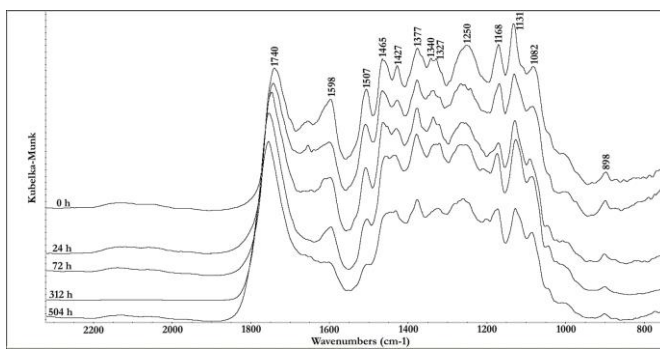


Figure 5

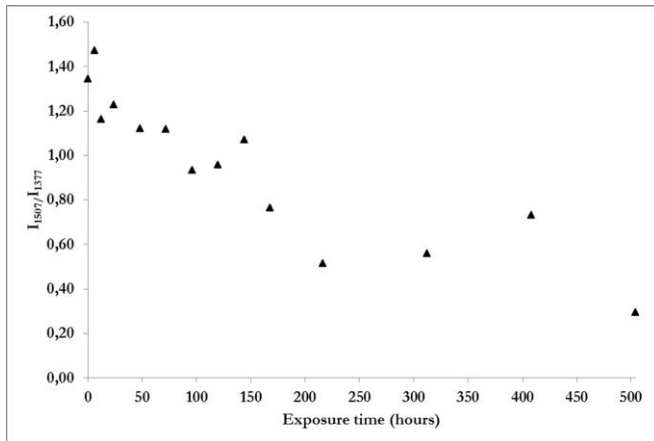


Figure 6

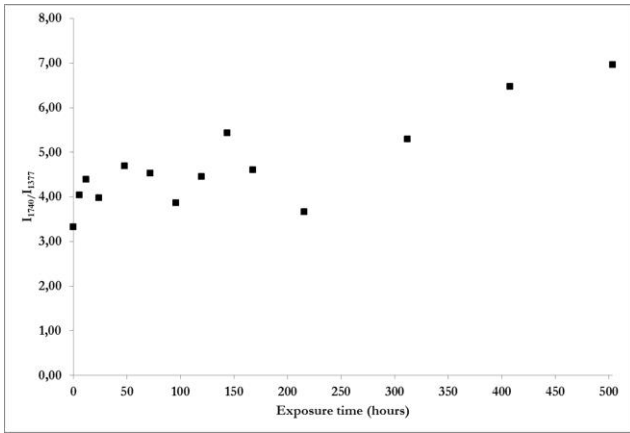


Figure 7

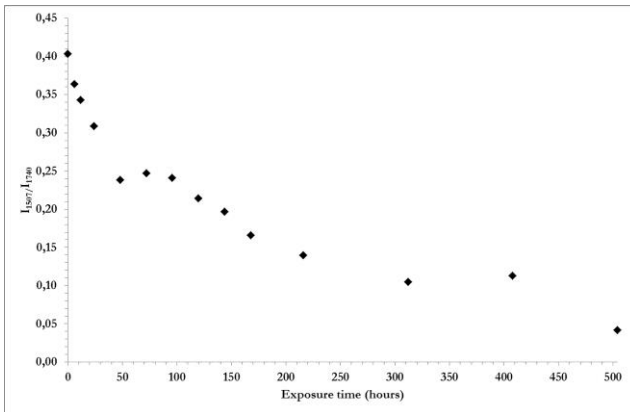


Figure 8

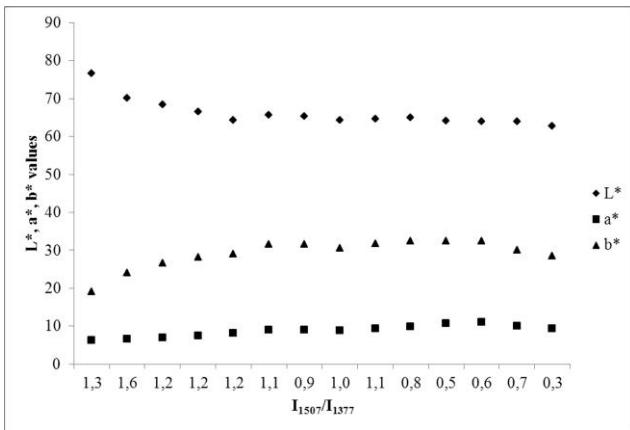


Figure 9

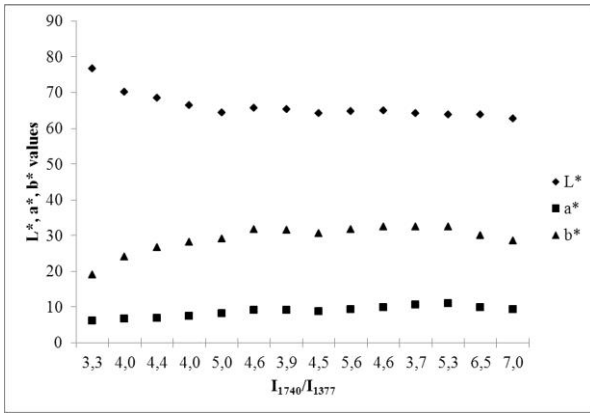


Figure 10

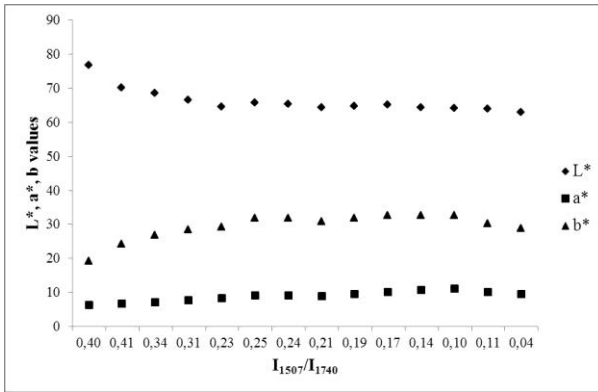


Figure 11

Table 1

Chestnut UT						Chestnut T							
Time (h)	L*	HSD	a*	HSD	b*	HSD	Time (h)	L*	HSD	a*	HSD	b*	HSD
0h	76.7	a	6.2	o,n	19.1	r	0h	62.7	d,c	9.4	h,g	34.5	d
1h	74.0	b	6.1	o	19.2	r	1h	63.4	b,a	9.2	i,h	32.0	i,h
2h	74.4	b	6.4	n	21.1	q	2h	63.5	b,a	9.1	l,i	31.7	l,i
3h	73.1	c	6.4	n	21.8	p	3h	63.6	b,a	9.0	m,l,i	30.9	p,o,n
4h	72.4	d	6.7	m	24.1	o	4h	63.2	c,b,a	8.9	m,l	30.5	q
5h	71.1	e	6.7	m	24.1	o	5h	63.6	b,a	8.8	n,m,l	30.6	q,p
6h	70.1	f	6.7	m	24.1	o	6h	63.5	b,a	8.8	n,m	30.6	q,p
7h	70.5	f	6.9	m,l	25.4	l	7h	63.8	a	8.9	n,m,l	30.6	q,p,o
8h	69.0	g	6.7	m	24.7	n	8h	63.5	b,a	8.8	n,m	30.4	q
9h	68.6	h,g	6.8	m,l	25.1	m	9h	63.6	b,a	8.8	n,m	30.6	q,p
10h	68.0	i	6.8	m,l	25.2	m,l	10h	63.7	a	8.8	n,m	30.6	q,p
11h	70.2	f	7.1	i	27.6	h	11h	63.6	b,a	8.8	n,m	30.6	q,p
12h	68.4	i,h	7.0	l,i	26.6	i	12h	63.4	b,a	8.6	n	31.0	n
24h	66.5	l	7.6	h	28.2	g	24h	63.1	c,b	8.6	n	30.8	p,o,n
48h	64.4	q,p	8.2	g	29.1	e	48h	62.8	c	8.9	m,l	31.3	m
72h	65.7	m	9.1	e	31.7	b	72h	62.3	e,d	9.1	l,i	31.5	m,l
96h	65.3	n,m	9.1	e	31.6	b	96h	61.8	f,e	9.5	h,g	32.4	g
120h	64.2	r,q,p	8.8	f	30.6	c	120h	61.2	g	9.5	g	32.1	h
144h	64.7	p,o	9.4	d	31.7	b	144h	61.3	g,f	9.8	f	32.7	g
168h	64.9	o,n	10.0	c	32.5	a	168h	60.5	h	10.3	e	33.7	f
216h	64.1	r,q	10.7	b	32.5	a	216h	59.3	i	10.8	d	34.1	e
312h	63.9	r,q	11.0	a	32.5	a	312h	58.6	l	11.8	c	36.1	c
408h	63.9	r	10.0	c	30.0	d	408h	57.1	m	12.4	b	36.5	b
504h	62.7	s	9.4	d	28.6	f	504h	55.0	n	13.3	a	37.1	a

Table2

Value	Intercept	Significance	time	Significance	time ²	Significance	Dummy (treated)	R ² adj
L*	70.39221	***	-0.03717	***	4.48x10 ⁻⁵	***	-6.19797	0.729
a*	6.843225	***	0.017939	***	-2.1x10 ⁻⁵	***	1.664813	0.736
b*	24.72024	***	0.04592	***	-7.2x10 ⁻⁵	***	5.230355	0.719