

Imaging of oxygen transmission in the oak wood of wine barrels using optical sensors and a colour camera

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Abstract

Background and Aims: Wine ageing in oak barrels is characterised by a series of physical and chemical processes, in many of which oxygen plays an important role. Therefore, to control better this phase of winemaking, it is not enough to know that oxygen penetrates into the barrel through the oak wood; it is also important to know how oxygen moves through the wood into the barrel, in what quantity and which factors are involved.

Methods and Results: We attempted to answer these questions with a high-resolution colour camera, ratiometric calibration and a planar optode with which the two-dimensional distribution and dynamics of oxygen within an oak barrel stave were studied. It was possible to make two-dimensional measurements when combined with microscopic optics and an appropriate camera, which permitted the measure of the transmission of oxygen into the barrel through the oak stave and the study of the mechanism of transmission. The trial was applied to both dry and wet oak wood, which revealed how the moisture content of the wood influenced oxygen transmission. The measurements were made in an airtight chamber under controlled temperature and pressure, which accurately reproduced the conditions of a stave in an oak wood barrel filled with liquid.

Conclusion: We were able to visualise the diffusion of oxygen in both dry and in wet French oak wood under conditions similar to those operating in a barrel. The moisture content of the wood influenced the oxygen transmission rate (OTR) through the wood.

Significance of the Study: For the first time, the dissolved oxygen dynamics and two-dimensional distribution of oxygen within the thickness of a barrel stave have been demonstrated. Wood water saturation is a key factor in the diffusion of oxygen through wood with barrel OTR decreasing during the ageing period.

Keywords: moisture content, oak barrel, oak wood, oxygen imaging, permeability

Introduction

Oxygen is an essential element in the ageing of wine in barrels, and for many years, there has been evidence that oxygen enters oak barrels and is transferred to the wine (Ribéreau-Gayon 1933). Few studies, however, have examined the entry of oxygen through the barrel during the wine-ageing process, with only two publications reporting the experimental measurement of oxygen entry into the barrel. The first (Vivas and Glories 1997) quantified the oxygen entry rate into two barrels by measuring the H_2SO_4 formed by SO_2 oxidation, where 2 mol H_2SO_4 are produced for 1 mol O_2 consumed. This measurement allowed the determination of the total oxygen consumed inside the barrel over 6 months and, furthermore, highlighted the impact of the geographic origin of the oak and the state of the oak (new vs used). This was the only study that established, by analysing two barrels, the route by which oxygen is delivered into the wine; the authors estimated that 21% of the oxygen content enters through the bung, 63% enters through the gaps of the staves and bases and only 16% enters through the oak wood. Apart from this study, only one publication (Vivas et al. 2003) was found in which the permeability of oak wood pieces to atmospheric oxygen under various conditions was reported. In this work, the influence of moisture content (MC) on the permeability of the wood to oxygen was measured; it was shown that the permeability of dry wood was between 20- and

100-fold higher than when moist, although the authors did not specify the MC of the oak. Singleton (1995) postulated, however, that the entry of oxygen into a barrel occurs only through the gaps between the pieces of wood from which the barrel is constructed and during the topping or racking processes. Singleton challenged the notion that oxygen enters through the wood, arguing that the oxygen that passes through the wood from the external environment would be consumed by the wine components impregnated into the wood or by oxidisable compounds in the wood, such as gallic acid and its derivatives, before reaching the wine inside the barrel. The oxidation products of these compounds would polymerise and form layers of brownish compounds, which have been observed on the interior of old barrel staves. In addition, Vivas and Glories (1996) demonstrated the role of ellagitannins as wine oxidation regulators and acknowledged their role as oxygen scavengers.

The measurement of dissolved oxygen (DO) using optical techniques has attracted increased interest in recent years primarily due to the decreasing cost of the electronic components, which has allowed the development of inexpensive, more compact and, above all, simple to operate equipment. These instrumental advances have promoted the development of new sensing materials for observing fluorescence quenching in the presence of oxygen (Wang and Wolfbeis 2012). Oxygen is normally measured at a point position on the sample, thus it is

necessary to use multiple measurement points to estimate the DO distribution in two dimensions (Nevares et al. 2010) to achieve an accurate representation of the entire sample. To measure DO at several positions in a material such as wood, Clark-type electrode micro-sensors (Del Hierro et al. 2002) have been used, in which oxygen diffuses from the point of insertion of the micro-sensor to the electrode through a permeable membrane. Micro-optode-type micro-sensors composed of optic fibres, in which one end is coated with a luminophore whose fluorescence is quenched in the presence of oxygen (Soriz and Hietz 2008), have also been used. Both systems are intrusive, altering the material structure under study and thereby preventing the collection of data over an extended period. The development of methods that can visualise specific molecules in physiological processes in real time using fluorescent optical sensors has improved our understanding of these types of materials, and this approach has become increasingly important in recent years (Tschiersch et al. 2012). The fundamental advantage of such methods is their wide spatial coverage (from mm² to cm²), their lack of intrusiveness, their high resolution and their capacity to collect measurements over a time scale ranging from seconds to days.

In this study, the two-dimensional (2D) spatial distribution and dynamics of DO were measured using oxygen imaging to characterise the oxygen entry of oak wood staves in a barrel. The measurement of the oxygen distribution at the interface formed by the width of the stave in contact with air on one side and the liquid content of the barrel on the other enabled the visualisation of oxygen transmission by diffusion through the oak wood and illustrated the importance of the MC in the wood to the final value of the oxygen transmission rate (OTR) through the oak staves.

Materials and methods

Equipment for the measurement of dissolved oxygen

The instruments used in this study were based on optical systems that take advantage of fluorescence quenching dynamics. The first instrument employed a planar oxygen sensor 40 × 40 mm² (SF-RPSU4; PreSens GmbH, Regensburg, Germany) coupled to a detection microscope [USB (universal serial bus) VisiSens (PreSens GmbH)] (Tschiersch et al. 2012). Although the VisiSens AnalytiCal 1 (PreSens GmbH) software controlled the camera settings, it was necessary to pre-calibrate the camera with respect to the sensitivity of the colour channel. The captured 24-bit and 1280 × 1024 pixel images (1.3 megapixel) were stored as portable network graphic files. The camera was placed 2 cm from the sensor with a field of view of 15 × 12 mm, generating a virtual resolution of 12 μm. The spatial resolution was 20 μm and was governed by the lateral diffusion of the oxygen sensor inside the sensing layer. The software managed the image acquisition and generated oxygen maps from the sensor response signal, for which the sensor was calibrated with two calibration points. The optical properties of the light source and the excitation and emission filters were adapted to the fluorescence response signals of the oxygen probe (Tschiersch et al. 2011). The second instrument selected for this work was the Fibox3 LCD Trace (PreSens GmbH), belonging to the Oxi-trace series, coupled with a trace oxygen sensor coated with PSt6 with an oxygen detection limit of 1 μg/L, 0–4.2% (PreSens GmbH). These two instruments enabled the non-intrusive measurement of the DO concentration. Major differences, however, exist in the ways in which the instruments employ fluorescence dynamic quenching (Kautsky 1939), which is the common principle of both. The instrument

employing the PSt6 sensor measured the delay between the emission and the detection of the luminophore signal, which is quenched by molecular oxygen. This quenching correlates with the oxygen concentration or the oxygen partial pressure at the sensor surface, which in turn is in equilibrium with the oxygen in the sample. Thus, this oxygen-imaging instrument exploits the correlation between the emission-reception intensity of the luminophore and the presence or absence of oxygen (Klimant et al. 1999). To avoid the inconsistencies that can arise from these types of correlation measurements (Kühl et al. 2007), a ratiometric calibration was used, in which the sensor element was composed of a luminophorous substance and a reference material, and both substances were fixed in a polymeric matrix to form a sensing layer. The interaction between the indicator molecules and the oxygen molecules quenches the red fluorescence of the dye, indicating energy transfer from the excited luminophore to the oxygen molecule during collision. As a result, the luminophore signal decreases with increasing oxygen concentration, while the signal of the reference substance is not affected by the oxygen and provides a signal of constant intensity. During the measurement, the reference compound and the luminophore are excited with the same light source [blue light-emitting diodes (LEDs) incorporated into the USB microscope] but emit light at different wavelengths. The luminophore emits red light, while the reference compound emits green light; these signals are captured by the red and green sensors, respectively, of the camera red, green and blue (RGB) LED chip in a single image. The DO was quantified using the ratio between the red and the green channel values of the captured RGB image. This method eliminates the primary interference sources due to the non-homogeneous intensity and colour of the blue LEDs, the variation in the luminophore concentration at the surface of the sensor and the difference in the thickness of the planar oxygen sensor surface. The oxygen concentration was calculated from this ratio by calibrating the sensor signal obtained from the exposure of the sensor to known oxygen concentration values. The linear Stern–Volmer model was incorporated into the software and revealed the oxygen concentration values in the images (Tschiersch et al. 2011, 2012). The instruments were calibrated by following the manufacturer's instructions, and prior to each assay, the calibrations were verified and corrected, if necessary, to account for potential sensor drift.

Measurement of the moisture content of wood

The MC of wood (with a minimum MC of 5%) was measured with the Brookhuis Micro Electronics MCM (Enschede, the Netherlands), a multichannel sensor with the ability to measure elevated resistance values. This instrument simultaneously measured 16 channels connected to electrodes, which, arranged in pairs, enabled the measurement of eight points in the range of 5–99% humidity at 20°C (100 GΩ to 10 kΩ) at a precision of ± 2%. The stainless steel electrodes were completely isolated electrically, except at one end for the connection and at the other end for the exact measurement in the wood insertion point. The electrodes were inserted into the wood by drilling a pilot hole and sealing the hole with epoxy to avoid the entry of moisture. For the resistance measurement, two electrodes with a separation of 3 cm were inserted at four depths (2.5, 5, 7 and 10 mm) from the side of the stave in contact with the liquid. For these measurements, another piece of wood from the same stave was placed contiguously to the piece used for oxygen image measurement. This stave piece was waterproofed on all sides except on its external (air side of the barrel) and toasted (liquid side of the barrel) faces, which were placed in contact

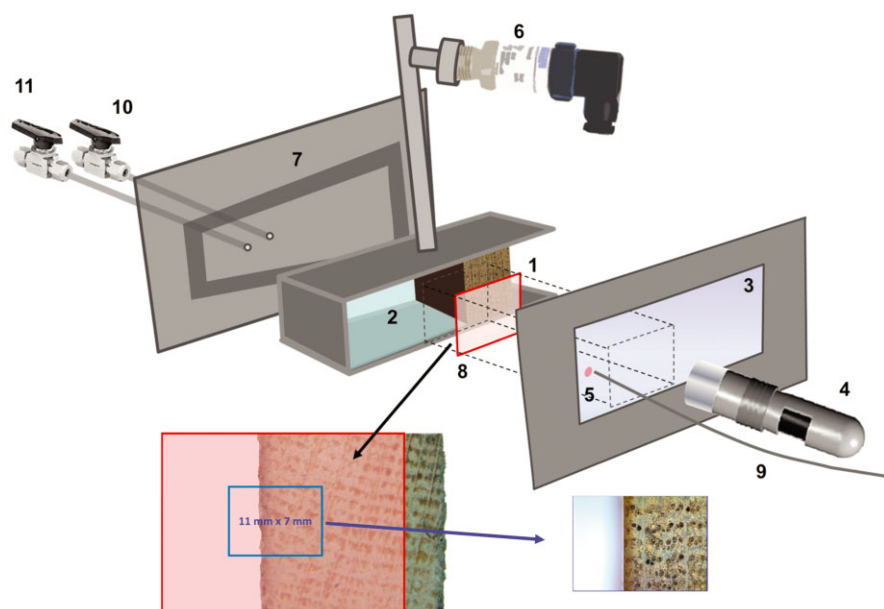


Figure 1. Schematic of equipment to measure the oxygen transmission rate across wood from the exterior to the interior of the stave under conditions similar to those operating in a barrel taking into account the wood water saturation. (1) piece of barrel oak stave, 27 mm thick; (2) liquid chamber; (3) glass; (4) USB microscope; (5) spot Pst6; (6) pressure transmitter; (7) stainless steel side; (8) planar optode; (9) optical fibre; and (10, 11) gas valves.

with water to monitor the MC. The calibration used was specific for oak (Fernandez 2013).

Experimental design

The permeability of wood to oxygen was measured with the device commonly used to measure gas permeability in membranes (ASTM 2005, 2007a,b), which typically comprised two chambers separated by the wood under study (The novelty in the experimental design stemmed from the manner in which the chambers were filled and the initial assay conditions were established. A pressure gradient governed by Darcy's law can be used, and the resulting gas flow is expressed in $\text{m}^3/(\text{m} \cdot \text{s} \cdot \text{Pa})$. Besides, the oxygen partial pressure difference at either side of the wood can be also considered, constituting a diffusion phenomenon. In the latter case, a molecular flow governed by Fick's first law is produced, expressed in m^2/s (Siau 1984). This is predominant in barrels because of the oxygen partial pressure gradient reached (Kelly and Wollan 2003). One of the chambers separated by the wood can be filled with air, and the other can be filled with nitrogen, establishing an oxygen partial pressure gradient similar to that produced in an empty barrel (Vivas et al. 2003, Sorz and Hietz 2006). To speed up the process, oxygen can be used instead of air, provided the flow rate of oxygen (the donor gas) and of nitrogen (the carrier gas) that are driven to the oxygen analyser is constant (Vivas et al. 2003). This design is commonly used for testing the permeability of layers (ASTM 2005, 2007a,b). The sample MC plays a decisive role in the oxygen diffusivity into the wood and must therefore be taken into account (Siau 1984, 1995, Vivas et al. 2003, Sorz and Hietz 2006). Therefore, a device developed by our group was built that reproduces the conditions inside a barrel, with liquid contacting the toasted interior face of the stave and the atmosphere contacting the external face of the stave (Del Alamo-Sanza and Nevares Domínguez 2012). This device also maintains the wood surface–liquid volume ratio similar to that present in the barrel. An oxygen measurement system employing PSt6 sensors was used to determine the wood OTR under the assay conditions. One side of the device is composed of a crystal that performs the DO reading using the optical systems (Figure 1). The device contains a stainless steel tube 40 cm in height with a pressure transducer (Transmitter S-10, Wika, Lawrenceville, GA, USA)

that measures and adjusts the hydrostatic pressure to that found in a stave in the middle of the barrel.

Assay procedure

A stave with a uniform structure and a distribution of the medullary rays parallel with the liquid contact surface of the stave in the barrel was selected from a French oak barrel (*Quercus petraea*) with medium toast. From this stave, a $40 \times 70 \times 27$ -mm piece was selected and fixed into the device with screws using the minimum tightening pressure necessary. The planar oxygen sensor was placed in contact with the liquid and the wood (Figure 1), and the spot PSt6 was attached to the centre of the transparent surface of the chamber full of liquid. The device chamber was tested to ensure air tightness by fixing a metal piece the same shape as the stave piece to the device in place of the wood. The device chamber was filled with N_2 , and the DO entry was monitored for 24 h. In addition, the pressure variation was corroborated with the pressure transducer. Once the tightness of the device was confirmed, and prior to each assay, the accuracy of the planar oxygen sensor (SF-RPSU4) in contact with the air (100% air saturation) was verified with the device chamber full of atmospheric air under the conditions of a climate-controlled barrel room (15°C , 80% humidity). All the air was then displaced with nitrogen (99.9992% Carburos Metálicos, Barcelona, Spain), and the device was maintained under these conditions for at least 2 h to de-gas the wood gradually from the interior to the exterior side of the oak stave. Afterwards, the device chamber was filled with pure water de-gassed with N_2 until a stable reading of 0% air saturation was achieved. Water was used instead of wine because the oxygen-consuming compounds of the wine will not permit the measurement of oxygen delivered to the wine. Water-soluble wood compounds capable of oxygen consumption, such as ellagitannins, are being considered to closely reproduce similar conditions to those operating in a barrel since otherwise the amount of oxygen reaching the wine through the wood could be overestimated. To reproduce the conditions under which greater oxygen transmission is produced in a barrel (Moutounet et al. 1994, 1998), the pressure inside the chamber full of liquid was adjusted by a syringe to a pressure of -100 hPa in the system's head space (upper part of the tube, next to the pressure

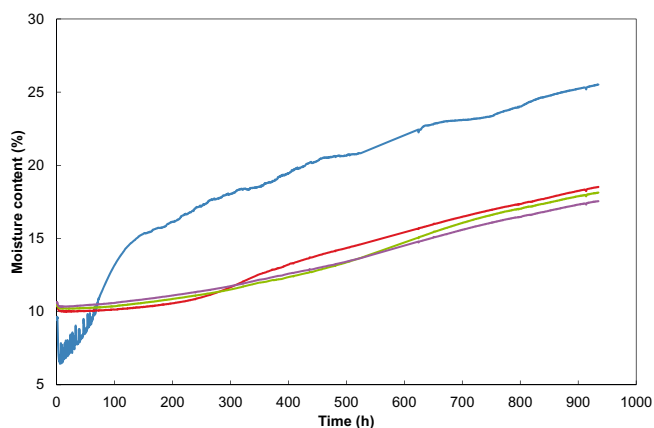


Figure 2. The increase in moisture content of a stave at a depth of 2.5 (—), 5 (—), 7.5 (—) and 10 (—) mm from the surface of the stave in contact with the liquid.

transducer). This is a common pressure inside the barrel during the wine-ageing process (Peterson 1976). This same piece of wood was studied for a total of 40 days, and its permeability to oxygen was consecutively analysed over periods of between 1 and 12 days depending on how fast the upper limit of the Pst6 sensor measurement range of DO was reached. Ten consecutive assays were performed with a cumulative wood–liquid contact time of 40 days allowing the MC to increase in the wood piece. Prior to each assay, the initial conditions of the de-oxygenated water to be measured were restored. In this way, it was possible to measure oxygen diffusion in the same wood piece but at variable MC because the MC of the wood increased considerably with time (Figure 2). This procedure ensured that the oxygen partial pressure gradient inside and outside the device never fell below 90% of the gradient found in a barrel (Piringer and Baner 2000).

The USB microscope was programmed to capture images every 10 min. These time points were alternated with those of the DO measurements by the luminescent system to avoid interference. Readings of the DO concentration in the liquid by the Pst6 dot were used to calculate the wood OTR for each assay under different wood MCs.

Simultaneously, in the same room and under the same temperature and humidity conditions, the evolution of the MC of the wood in contact with the water was measured in duplicate as stated above.

Image processing

The images were processed with the software AnalytiCal-1 by transforming the captured RGB images into ratiometric images and subsequently converting them into calibrated images in which the colour indicates the DO content of the area in the photograph (Figure 3). These calibrated images were imported with the free software ImageJ (<http://rsbweb.nih.gov/ij/>), and all images were converted to interactive three-dimensional (3D) surface plots in which the vertical axis represented the DO concentration (Figure 4). These images were rotated to observe the oxygen profile within the thickness of the stave and within the liquid at different times in each test.

The round front shown in the wood is due to the illumination of the circular disposition of the LEDs around the lens in the camera (Figure 3). The ratiometric referenciation of heterogeneous light field is a core functionality of VisiSens. In the resulting ratiometric images, intensity-based interferences should be suppressed. We should however be aware of some constraints.

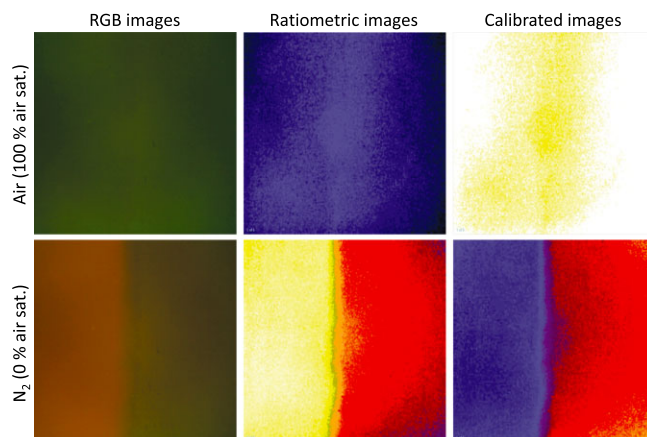


Figure 3. Captured [red, green and blue (RGB)] and processed (ratiometric and calibrated) images during calibration for each of 100% air saturation (top panels) and 0% air saturation (bottom panels).

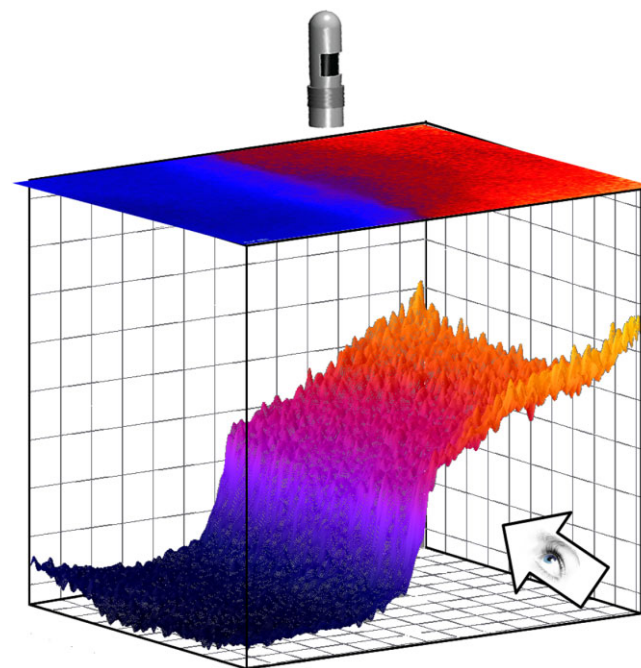


Figure 4. To study the dissolved oxygen (DO) profile, a three-dimensional (3D) image was created where the vertical axis represents the DO level. There is a sharp change in the border wood–liquid DO because the liquid was deoxygenated at the beginning of the test. If the 3D image is rotated, the DO profile can be seen within the thickness of the stave.

Because of better signal to noise ratio in the middle of the image the colour pattern may be ‘smoother’ in this region. The choice of minimum and maximum could accentuate even marginal heterogeneities. VisiSens uses an interference notch filter that blocks the yellow part of the spectrum. This is essential when measuring with (minor) light interferences, for example room light, but when measuring through highly reflective surfaces, glass in this work, this filter can eventually cause a pattern in the middle of the image, as has happened.

Results and discussion

In the first assay (0 h), the stave was tested before coming into contact with the liquid, which represents the first few minutes of the operation of staves of a new barrel that has just been filled

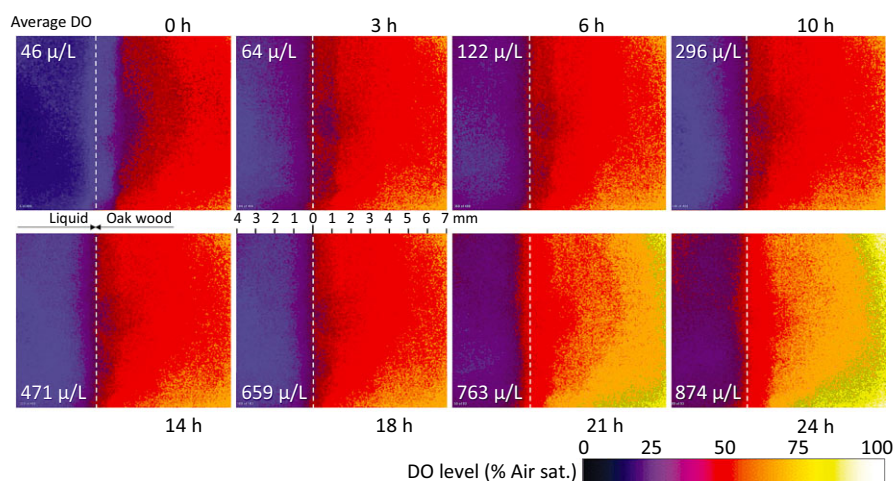


Figure 5. Dissolved oxygen (DO) distribution at eight time points during a 24-h period, where the stave was tested before coming into contact with the liquid (time = 0 h). The white vertical line represents the liquid /wood border.

(Figure 5). It is evident that the oxygen content of the wood decreased from the equilibrium concentration in the presence of atmospheric oxygen (white-yellow; Figure 3) to a value of approximately 50% of the air saturation (red tone) due to the de-gassing performed at the beginning of each test. Similarly, the wood area affected by the toasting process (usually between 1 and 5-mm thickness) was in equilibrium with the deoxygenated water, and the first millimetre of wood in contact with the liquid therefore exhibited blue tones representing a DO value lower or equal to 15% of the air saturation. These observations are corroborated by the values of the MC variation as a function of the wood–water contact time measured simultaneously in the contiguous stave piece (Figure 2). The wood region affected by the toasting process (2.5 mm) exhibited an initial MC of 7%, while the remainder of the wood exhibited an MC of 10–10.5% (Figure 2). Additionally, the MC evolved differently in the region affected by toasting compared with the remainder of the stave wood. This result suggests that the structure of the toasted wood was modified during toasting and that the MC decreased, which implies that oxygen diffusivity is greater in drier wood (Sorz and Hietz 2006). This result coincides with the data obtained by Hugi et al. (2007), in which the extent of toasting affected the permeability of the oak wood to oxygen.

After 3 h of contact between the wood and water (Figure 5), the DO distribution began to change. In addition, a significant increase in DO was observed in the toasted wood region transforming from blue to red tones, and the DO concentration of the liquid layer in contact with the wood also increased to a lesser degree, evolving to a purple colour. It was observed that the total DO content of the liquid increased as the oxygen released from the wood was incorporated, as evidenced by the transition from blue tones to reddish tones in the image from the sensor which was in contact with the water. This increase in the oxygen content of the liquid, as observed at the eight collection times shown in Figure 5, did not arise due to the oxygen release or outgassing of the wood, but rather due to the oxygen from the atmosphere. This conclusion is supported by our observation that the concentration of DO increased in the same manner in both the stave and in the liquid.

Because the DO concentration of the air surrounding the exterior side of the wood (not collected in the images) is constant and equal to 100% air saturation, the oxygen in the liquid inside the chamber resulted from the formation of a DO gradient from the atmospheric air to the liquid contained in the chamber, as observed in the DO profile. Therefore, the oxygen permeation was a mass-transmission process originating from a concentration gradient, which is the gradient of oxygen partial pressure

that gives rise to the diffusion of a gas in a porous solid as governed by Fick's law.

In the second assay, the same wood piece was used after being placed in contact with water for 48 h (Figure 6). The de-gassing process was performed once again, as described in the Materials and methods section. In contrast, however, to the pronounced effect of outgassing on the dry wood, the DO difference across the toasted wood interface thickness and the remainder of the wood following de-gassing was subtle. Likewise, the de-gassing process for the rest of the wood included in the image was less efficient. Evidently, the MC of the wood was modified after being held in contact with the liquid for 48 h. Furthermore, it is known that the oxygen diffusivity in the stave decreases with increasing MC (Sorz and Hietz 2006). The DO profile evolved with time, although in a manner less pronounced than that observed for the wood that was not previously in contact with water (dry oak wood). In this case, the DO profile reflects an increase in the DO concentration across the thickness of the wood and the adjacent liquid. Once again, the observed oxygen resulted from the external air, and the increase represents a diffusion process due to the difference between the oxygen partial pressure of the external environment (100% air saturation) and the liquid inside the barrel (less than 10% air saturation).

The DO measured with the Pst6 spot in the liquid (Figure 1) allowed the oxygen that was transferred into the liquid through the wood to be quantified and its evolution with time to be determined. As previously mentioned, the use of pure water, which, in contrast to wine, does not consume oxygen, allowed the mass balance and the rate of oxygen entry into the liquid through the wood to be determined. This calculation provided the OTR value, which in cooperage is usually expressed in milligrams of DO per litre of liquid over 1 year of entry into the barrel. Figure 7 shows the calculation results for some of the assays performed on the analysed wood piece and clearly reveals that the contact time of the wood with the liquid in the chamber affected the decrease in the wood OTR. This effect was also observed by Vivas et al. (2003) in wood pieces that were less thick than that of the staves and that were not in contact with liquid.

In the assays performed at different liquid contact times (0, 2, 7, 12 and 40 days) on the same stave piece, the MC along the first 5 mm of the wood depth depended on the contact time with the liquid. From 5 mm to 15 mm of depth, the MC of the wood increased uniformly from 10 to 18% after 40 days of humectation (Figure 7b). During the first 48 h, the MC in the first 5 mm of depth was less than 10% because the toasted wood

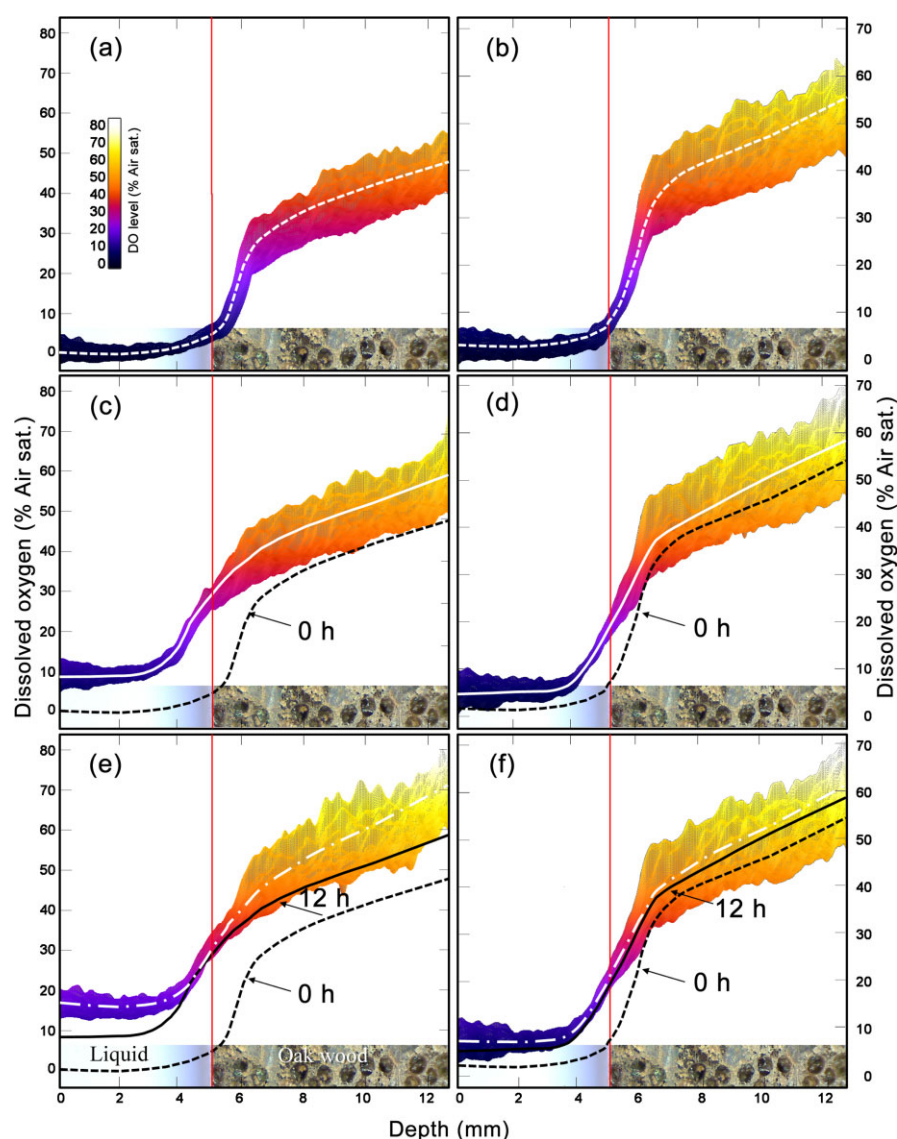


Figure 6. Dissolved oxygen (DO) profile after (a,b) 0, (c,d) 12 and (e,f) 24 h of the oak piece and the liquid in the chamber for the (a,c,e) dry oak wood and (b,d,f) the same oak that had been in contact with water for 48 h. The coloured band expresses the range (min.–max.) of DO at different depths of the wood. The red vertical line represents the liquid/wood border. In order to see the evolution of the average DO profile they have been maintained in the subsequent parts of the figure so the profile evolution with time can be compared (a,b) 0 h; (c,d) 12 h and (e,f) 24 h; the colour of line is t for improved visualisation.

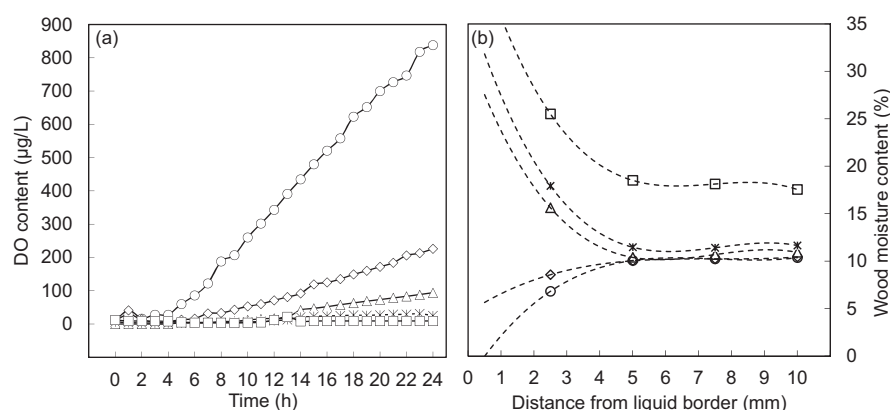


Figure 7. (a) Effect of time of oak in contact with water on the transmission of oxygen [as measured by dissolved oxygen (DO)] over 24 h through the oak piece and (b) on the moisture content of the oak piece measured from the interface of the liquid and the oak piece. (a) For selected assays performed on the analysed wood piece of contact times for the oak with water range from 0 (○), 2 (◇), 7 (△), 12 (×) and 40 (□) days with OTR calculated from the slope of the different rates as 304, 76, 32, 9 and 5 mg/(L · year), respectively.

layer did not reach saturation, making it much more permeable to oxygen. After the first 2 days, the humectation of these first layers became evident (Figure 7b) and the permeability to oxygen decreased considerably. This behaviour was observed because the impregnation front forms and is located within the

first 5 mm for staves 25 mm in thickness (Feuillat 1996). Across the depth of the liquid front (mm), the MC of the wood exhibits a free water state because it has reached the fibre saturation point (FSP), which, for oak under the assay conditions of 15°C and 85% RH, results in a MC of 29% (Kollmann and Côte

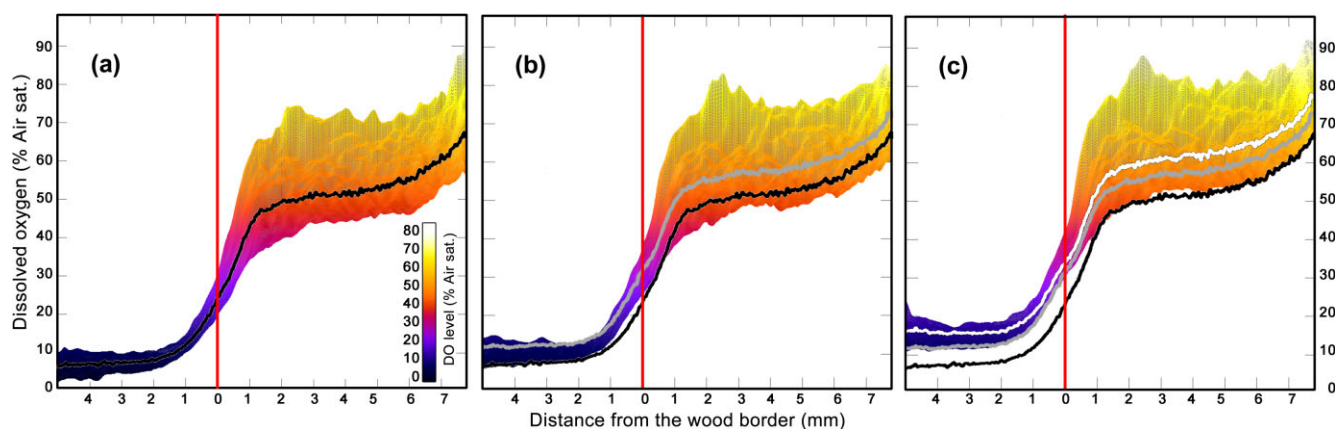


Figure 8. Dissolved oxygen (DO) profile throughout the surface of the stave as a function of the stave position and in the liquid as a function of the distance to the wood in the same wood piece after 40 days of liquid contact. The red vertical line represents the liquid /wood border.

1968). In the remainder of the wood, the moisture remains in a hygroscopic state, that is fixed to the wood cellulose, hemicellulose or lignin (Siau 1984). The models developed by the Ruiz de Adana group (Ruiz de Adana et al. 2005) predict that the impregnation kinetics of the oak wood staves that comprise the barrels will slow from day 40. At 40 days, the stationary phase is initiated, and 82 days are necessary for the MC in the exterior part of the stave to increase after it contacts the liquid in the interior face (Ruiz de Adana 2002). Across the entire stave thickness (25 mm), gas transport is governed by Fick's law (because there is no pressure difference between the inner and outer faces of the stave), and although in the first 5 mm of the stave the moisture is in free form, the oxygen flow is negligible according to Darcy's law. Thus, because the first few millimetres of the inner part of the stave are in contact with the liquid, the oxygen diffusion is governed by the oxygen diffusivity in water; in the remaining fraction of the wood, the diffusion is governed by the oxygen diffusivity in air, with the oxygen diffusion coefficient in saturated air 10820 or 10.82×1000 times greater than that in water at 15°C (Denny 1993).

The assays performed after 7 and 12 days of humectation revealed the same dependence of the OTR on the MC of the wood, although in a more pronounced way, as in both assays the FSP reached the inner face of the stave. Finally, an assay was performed on the wood after 40 days of stave–liquid contact, which yielded extremely low OTR values (Figure 7a). This result revealed that the wood OTR decreases with increasing MC of the wood. This decrease can explain the loss of 98% of the OTR because, in the last assay, the wood exhibited an OTR of 5 mg/(L · year) (Figure 7a), which deviated significantly from the initial value of 304 mg/(L · year). After analysing the OTR of this wood sample for 12 days following 40 days of humectation, it was observed that although the oxygen diffusion was slower, which produced a lower oxygenation rate (Figure 7a), the oxygenation did not cease. This result indicates that even when the stave wood reaches the FSP in the area that was in contact with the liquid, the oxygen flow from the external environment to the liquid still occurs. Thus, we confirmed that oxygen permeation continues in wood when a portion of its MC is in the free form.

At the beginning of the assay (day 0), after initiating the outgassing protocol, the first few millimetres of the stave in contact with the liquid (Figure 8) experienced less effective outgassing than the previous samples (Figure 6). In that region, the moisture in the wood is in its free form, and the oxygen diffusivity decreased dramatically after the beginning of the

experiment when only hygroscopic water was present (Figure 7b). Because a portion of the toasted wood was saturated with water, the stave permeability as a whole was slower and the evolution of the DO along the wood had to be observed over a scale of days rather than hours. At 6 days, the DO profile had increased, indicating that oxygen evolution was occurring by diffusion (due to the differences in oxygen partial pressure or concentration) from the external environment towards the liquid; the external oxygen remained at 100% air saturation, whereas in the liquid, it was only 7%. After 6 days, the DO reached an average value of 12%, and after 10 days of being in contact with the liquid, it reached 16% (Figure 8).

According to the work of Feuillat (1996) and Ruiz de Adana (2002), the impregnation front of a stave in equilibrium is 4- to 5-mm thick. Therefore, the remainder of the wood will not reach the FSP, but rather exhibit varying MC in the hygroscopic form. The saturated part of the inner face of the stave in contact with the liquid, which includes the wood affected by the toasting process, contained moisture in the free form. The thickness of the wood stave can be approximated as a multilayer system differentiated by its MC. Under these conditions, for a multilayered system with n layers, a series resistance model (often called the ideal laminate theory) is often used to produce a steady-state flux equation (Graff et al. 2004). Further, it is often more convenient to use the transmission rate (TR) instead of permeability (P), which is described as the quantity of the permeate passing through a membrane per unit of area per unit of time at the steady state. To understand the permeation through a multilayer stack, it is necessary to know both the solubility and diffusivity of each constituent layer, which in this case depend on the water/air oxygen solubility and diffusivity. According to this approach, the transmission rate is equal to the lower of the individual rates of each layer. Moreover, according to this approximation, the limiting layer of a stave is equivalent to that of wood immersed in water, for which the diffusivity is that of oxygen in water, which is lower than the diffusivity in air. Because the remaining wood layers do not reach the FSP and the water is in the hygroscopic form, the OTR depends on the oxygen solubility in the air. Therefore, the OTR will reach its minimum value when the water saturation front reaches a maximum, which, according to the work of Feuillat (1996), will occur 82 days following the filling of the barrel. The front will exhibit a 4- to 5-mm thickness.

These results reveal the importance of the level of wood water saturation to the OTR. It was deemed appropriate to use a system approximating the conditions in a barrel

(liquid–wood–air). This protocol (Del Alamo Sanza and Nevares Domínguez 2012) was therefore used in this work to evaluate the OTR in barrels. In the work of Vivas et al. (2003), a gas–wood–gas system was used following the guidelines established for assays of membrane permeability. Such a protocol did not reproduce the actual conditions of staves in a barrel, as the water saturation effect was not considered.

Conclusions

Oxygen imaging is a valuable technology for studying oxygen dynamics in wood and has enabled the visualisation of oxygen transport through water saturated humid wood, confirming that French oak wood is permeable to oxygen. When the wood contains water in its free form, oxygen contribution to the liquid that contacts the stave is observed, confirming that oxygen diffuses from the external environment and is not a product of the wood de-gassing process.

The OTR across wood from the exterior to the interior of the stave was measured under conditions similar to those operating in a barrel taking into account the wood water saturation, which is a key factor in the diffusion of oxygen through wood.

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