



NOTE

Open Access



# Origin and characterisation of the extractable colour of oak heartwood used for ageing spirits

Nicolas Vivas<sup>1\*</sup>, Marie-Françoise Bourden-Nonier<sup>1</sup>, Nathalie Vivas de Gaulejac<sup>1</sup>, Claire Mouche<sup>2\*</sup> and Cybille Rosy<sup>2\*</sup>

## Abstract

In this study, investigation is focused on the soluble part of oak heartwood colour, with the objective of defining its impact on the colour of spirits after a barrel ageing. Colour is an important parameter for estimating the quality of many beverages and food items. Acetone with 30% water permitted a fast and complete extraction of the soluble colouring matter of heartwood. Water is necessary to improve the extraction of wood fibres by hydration. In heartwood, the repartition of colour is heterogeneous. The outer heartwood, which is new wood, presents a lower soluble colour than the inner heartwood, which is older. This difference is due to the polymerisation by oxidation of the ellagitannins during the natural process of wood ageing and the copolymerisation of cell-wall polymers as polysaccharides.

**Keywords:** *Quercus*, Colour, Heartwood, Ellagitannins, Oxidation, Polymerisation

## Introduction

Colour represents the first contact with a material. Generally, the importance of colour in the quality evaluation of many products is acknowledged. Concerning the particular issue of oak, the colour of the wood has a direct influence on the evaluation of its quality and price [1–4]. For wine or spirits ageing, oak wood is often used for making barrels [5]. During storage, a part of the colouring matter is solubilised and impacts the colour of the beverage [6]. Knowledge of the compounds responsible for wood colour and its chromatic properties are of interest; however, despite the importance of the subject, very few studies on it have been conducted.

Originally, wood colour was thought to be an accident caused by the development of microorganisms on living trees, oxidative reactions or reactions of metal on cut trees [7]. Polyphenols, specifically tannins, have a

key role on wood colour evolution through oxidative chemical or biochemical reactions [8]. However, oak also presents a natural colour, which is clear and light in sapwood and dense and dark in heartwood. The colour gradually changes in the heartwood from a clear yellow to a reddish-brown as a function of wood age; therefore, wood from young trees have lighter colour than wood from older trees [9]. The intensity and nature of the colour cannot be totally explained by the concentration of polyphenols and ellagitannins [10]. The situation is complex and involves the chemistry of ellagitannins in oak wood as the tree ages. During the sapwood–heartwood transition (duraminisation), the wood accumulates large amounts of water-soluble extract that correspond to the amount of polyphenols, specifically ellagitannins [11, 12]. In the second step of the heartwood ageing process, the ellagitannin contents decrease regularly while ellagic acid increases with hydrolysis reactions; however, in the first 20 years of ageing, the dimeric ellagitannins increase transitorily [12]. Apart from hydrolysis, the decrease in ellagitannins is also due to polymerisation [12] and insolubilisation of ellagitannins [11].

\*Correspondence: nicolas.vivas@u-bordeaux.fr

<sup>1</sup> Demptos Research Center, Bordeaux University, Institute of Molecular Sciences, 351 cours de la Libération, Talence, 33405 Bordeaux, France

<sup>2</sup> Bordeaux University, Institute of Molecular Sciences, 351 cours de la Libération, Talence, 33405 Bordeaux, France

Soluble oak wood colour is less well documented. The compounds responsible for the darker colour of old heartwood are due to the presence of coloured extractives [13]. The cold-water-soluble fractional decrease from recent to old heartwood colour is from lightness to redder and yellower. The main part of the soluble form of colour can be found by applying gel permeation chromatography of the dry acetone–water oak extract to polymeric ellagitannins with an average Mr of 4000 Da [13]. More recently, an attempt to partially characterise the coloured soluble polymeric ellagitannins from oak was published [14]. The polymeric chromophores presented the same colour as described in previously cited articles, with an average Mr of  $2700 \pm 375$  Da. The formation is probably due to oxidation by Fenton's radical oxidation during heartwood ageing. Using in vitro conditions, a synthetic coloured soluble polymeric ellagitannin are reproduced by oxidation and confirmed the origin of the natural polymers formed in the wood.

In this study, the colour characteristics and intensity of extractable oak wood polymers and the solubility properties and solvent composition is presented as well as a clarification of oak wood soluble colour definition.

## Materials and methods

### Standard products

Solvents and chemicals were supplied by Sigma-Aldrich™ (Saint-Quentin Fallavier, FR), and ethanol was supplied by VWR Chemicals™ (Dublin, IR). Both gallic acid and ellagic acid were supplied by Acros™ (New Jersey, US); castalagin and vescalagin were purified from *Q. robur* heartwood under conditions described by Vivas et al. [15]; castalin and vescalin were produced in vitro with previously purified castalagin and vescalagin as described by Vivas et al. [16]; and dimeric ellagitannins were isolated with the procedure of Scalbert et al. [17].

### Plant material

The oak samples contained *Quercus petraea* and *Quercus robur* heartwood that was approximately 175 and 130 years old, respectively, from the homogeneous and appropriately maintained centre of France's forest compartments. Only the first quarter of the trunk, the cooperage grade timber, was used for the study. A visual evaluation of the colour allowed for the elimination of the sapwood (the first 25 rings) and the collection of the outer heartwood (30 to 75 rings) and the inner heartwood (75 to 120 for *Q. robur* and 75 to 165 rings for *Q. petraea*). We rejected the sapwood–heartwood transition (the first 26 to 29 rings) and the heart of the trees (121 to 130 and 166 to 175 rings for *Q. robur* and *Q. petraea*, respectively). The wood was chopped and naturally dried for 24 months. Different samples were planed

and then crushed to sawdust in liquid nitrogen before being strained to keep only the particles smaller than a 60-mesh size. The samples were freeze-dried, stored and analysed within a period of 2 months. Different samples are collected and represented 100 g of dry wood, each corresponding to 3 different trees for each species.

### Polyphenols and colour extraction

One gramme of sawdust (60 mesh) was extracted with 100 mL of an appropriate solvent or a mixture of solvents for 12 h at room temperature (20 °C) in the dark and during stirring (150 rpm). For the specific experiment on pH influence, values of pH are measured with a Orion 3 star and electrode Orion 8107 Numd equipment (Thermo Scientific, Chelmsford, USA) and adjusted on the different solutions by acetic acid (N concentration in water) for decreasing pH or NaOH for increasing (N concentration in water). For temperatures essay a drying oven is used (Thermostatics M 30-750, Memmert GmbH + Co.KG, Schwabach, Germany). For the fractionation and characterisation of the extractable colour investigation, samples extraction with an acetone/water (7/3, v/v) mixture is specifically used. The resulting extract containing polyphenols and colour was then filtered through 0.45- $\mu$ m membranes and freeze-dried before analysis.

### Colour measurements

Absorbance measurements at 420 nm (yellow hue) were made directly on the extraction solution. All optical densities made at 420 nm were calculated for 1 g of dry extract. The spectrophotometer is an Anthelie Secomam™ system (Champigny sur Marne, FR).

The CIE  $L^*$ ,  $a^*$ ,  $b^*$  chromatic characteristics were measured on a CIELAB Konica Minolta 508™ apparatus (Carrières-sur-Seine, FR).  $C^*$  the saturation and  $h^*$  the hue angle obtained by calculation:  $C^* = (a^{*2} + b^{*2})^{1/2}$  and  $h^* = \text{atan } b^*/a^*$ .

Colour analysis is conducted in triplicates for each 3 different trees for each species. Average variability (3 trees  $\times$  3 measures) represented 9% for colour parameters CIE  $L^*$ ,  $a^*$ ,  $b^*$  and 12% for O.D. 420 nm.

### Phenols estimation

To determine the total polyphenols index, the absorbance of water-diluted sample extracts is recorded at 280 nm [18]. Correct dilution are estimated for a final maximum absorbance lower than 1.5. After correction of the dilution, results were expressed as optical density per g of dry wood.

### Fractionation and analysis of extractable heartwood polyphenols and colour

A two-step procedure for the fractionation of the extractable inner and outer heartwood colour is applied. Prefractionation of the total freeze-dried acetone/water extract by low-pressure chromatography was performed on a Sephadex LH-20–100 column (20 × 2 cm, flow rate 3.5 mL, detection 280 nm for phenols and 420 nm for the colour). 3 fractions were obtained as follows: Fraction I, by water elution; fraction II, by 30% vol. MeOH; and fraction III by 100% vol. MeOH. The characterisation of the molecular weight and the identification of the main compounds were determined by the size-exclusion chromatography (SEC) of the acetylated fraction. Freeze-dried fractions I, II, and III of 10 mg each were acetylated with pyridine–acetic anhydride (1/1, v/v) for 3 days at room temperature. The precipitate was obtained by pouring the mixture into cooled water and recovered by centrifugation; it was then washed with distilled water, MeOH and finally chloroform. The precipitate was dried and dissolved in 0.5 mL tetrahydrofuran (THF) and filtered before the SEC analysis. The SEC analysis was performed as described by Vivas et al. [14] using a Thermo Quest™ instrument equipped with three columns (300 × 7.8 mm, TSK™ Gel 1000 HXL, 2000 HXL and 2500 HXL in series), calibration curve was obtained with polystyrene standards and as eluent THF. Experiment is conducted for a triplicate series of oak heartwood, the total variability of the fractionation method represent ± 12% for the dry weight and 7% of the colour measurements.

### Partial characterisation of heartwood coloured polyphenols

Pyrolysis/GC/MS were performed on a ISQ Thermo Scientific™ instrument. Samples (approximately 0.5–0.6 mg) were placed in the pyrolyzer and duplicate pyrolysis experiments were carried out at 500 °C. General profiles for pyrolyzates were obtained using EI-MS. Separation of the pyrolysis products was achieved using a fused-silica capillary column: DB-5MS™ (30 m × 0.25 mm i.d × film thickness 1 µm). Helium was used as the carrier gas at a nominal flow rate of 1 mL/min. The injector was at 250 °C in the splitless mode. The gas chromatography oven was operated using the following programme: isothermal for 1 min at 40 °C, then raised from 150 °C at 4 °C/min and raised to 300 °C at 15 °C/min for 5 min. The mass spectrometer was set at 70 eV. Different GC peak are identified by standard sample injection, GCMS library when spectra are available and comparison with a previous work [19]. THM/GC/MS were performed with the same instrument. Tetramethylammonium hydroxide (TMAH, 25% aq., 5 mL) was added to the samples (approximately 0.5–0.6 mg). The gas chromatography oven was operated

using the following programme: isothermal for 1 min at 50 °C, then raised from 300 °C at 8 °C/min for 5 min.

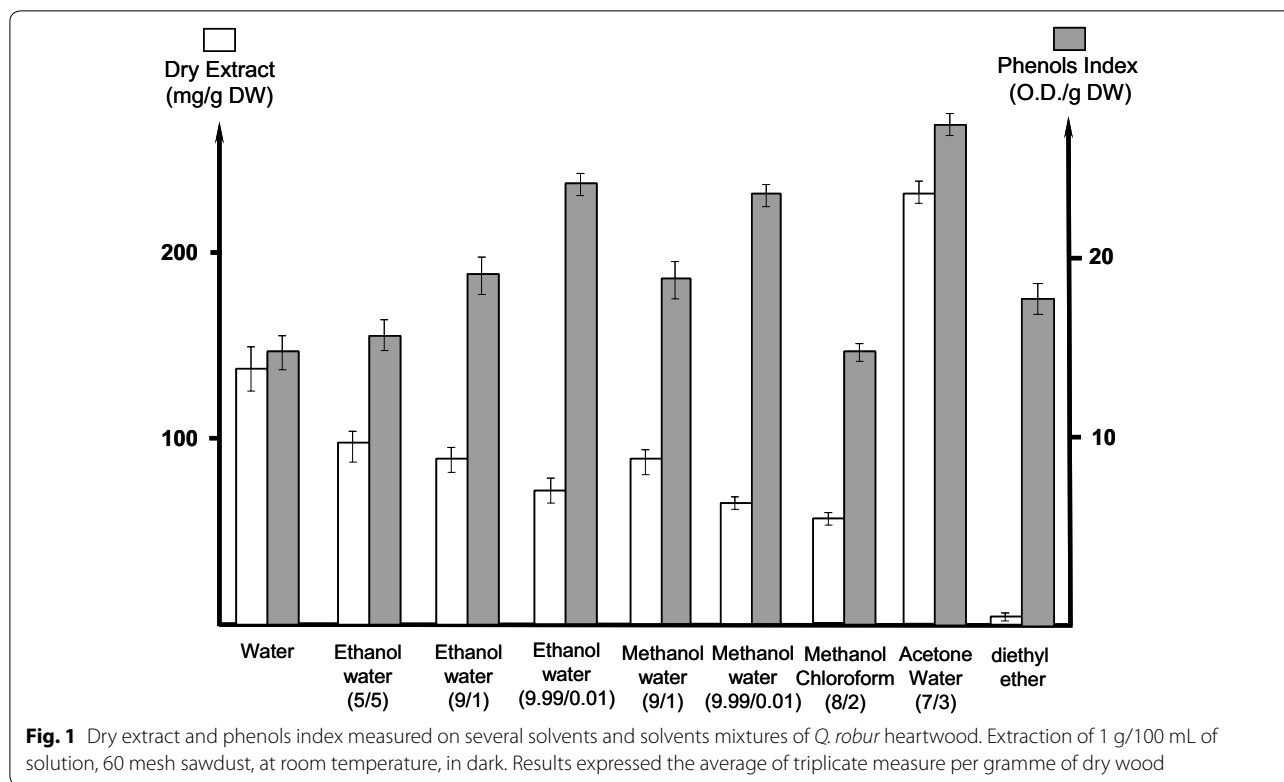
NMR spectra (<sup>1</sup>H, HSQC and HMBC) of compound were performed at 298 K on a Bruker Avance III 600 MHz spectrometer equipped 5 mm BBI probe with z-gradient. <sup>1</sup>H-NMR (32 scans) experiments was obtained at 600.16 MHz with chemical shifts reported in ppm downfield from tetramethylsilane (TMS) as international standard. NMR sample was prepared by dissolving about 10 mg of powder in 500 µL of methanol-d<sub>4</sub> (CD<sub>3</sub>OD, D = 99.96% Euriso-top).

## Results

### Heartwood soluble colour and dry extract

To compare the effect of different solvents and mixtures of solvents on the dry extract and the phenols of heartwood extracts, an experiment are conducted (Fig. 1). Considering the amount of dry product extracted from the heartwood, the water and acetone–water mixture provided the best results. Considering the amount of phenols, mixtures of ethanol–water (9/1), methanol–water (9/1) and acetone–water (7/3) allowed for higher phenolic extraction. Diethyl ether extraction produced a lower dry extract but was richer in phenols. Methanol–chloroform (8/2) had a lower polarity of solvent–mixture boundary extraction of the wood extract. Temperatures of 80 °C for water and 40 °C for ethanol–water or methanol–water (5/5) mixtures improved the dry extract but had a weaker effect on phenols. For 80 °C dry extract increasing to 42%, but phenols reach only 12% more, with some high risk of structure thermodegradation; for 40 °C on ethanol–water or methanol–water (5/5) dry extract increasing to 65%, but phenols improve no significantly to 6%. Increasing the pH from 3–4 to 8–10 presented a similar effect but had problems with extract composition stability; after half hour in room temperature, extracting solution became brown with precipitation of phenols. Acetone–water (7/3) presented a good compromise for dry extract and phenols content.

The colour parameters used on heartwood extracts measurements are represented firstly by an optical density at 420 nm, the maximum absorbance of visible range spectra for heartwood extracts and secondly by the CIE *L\*a\*b\** system (Table 1). The extract solvents and mixtures affected the intensity and nature of colour. With the exception of diethyl ether, water and other mixtures of solvent extracted a significant amount of heartwood colour. Using diethyl ether on *Q. robur* or *Q. petraea* extracts presented a lower A<sub>420</sub> nm, a higher *L\** lightness and a higher *b\** yellower than with water and other solvent mixtures. The acetone–water (7/3) solvent mixture had a darker colour extract with *L\** near 25 ± 1.5%. The level of ethanol in the solvent mixture affected the



**Table 1** Colour parameters of *Q. robur* and *Q. petraea* heartwood extracts. Incidence of different solvents or mixtures of solvents

	<i>Q. robur</i>						<i>Q. petraea</i>					
	A <sub>420</sub> <sup>a</sup>	L <sup>*b</sup>	a <sup>*b</sup>	b <sup>*b</sup>	C <sup>*b</sup>	h <sup>*b</sup>	A <sub>420</sub> <sup>a</sup>	L <sup>*b</sup>	a <sup>*b</sup>	b <sup>*b</sup>	C <sup>*b</sup>	h <sup>*b</sup>
Water	3.6	46.6	6.3	21.1	22.0	1.3	3.5	49.5	5.4	19.2	19.9	1.3
Ethanol–water (5/5)	4.1	42.0	7.1	23.5	24.5	1.3	3.9	46.7	6.8	22.6	23.6	1.3
Ethanol–water (9/1)	4.7	37.6	10.1	24.9	26.8	1.2	4.9	32.4	10.4	26.5	28.4	1.2
Methanol–water (9/1)	4.6	38.2	9.8	25.2	27.0	1.2	4.5	31.8	10.1	24.3	26.3	1.2
Acetone–water (7/3)	5.7	27.3	5.2	11.5	12.6	1.1	5.6	25.8	6.2	13.2	14.6	1.1
Diethyl ether	2.2	61.4	3.4	29.4	29.6	1.4	2.6	58.7	2.6	28.7	28.8	1.5

Extraction of 1 g of dry wood for 100 mL of liquid, at room temperature and dark

<sup>a</sup> O.D. at 420 nm, value calculated for 1 g of dry extract

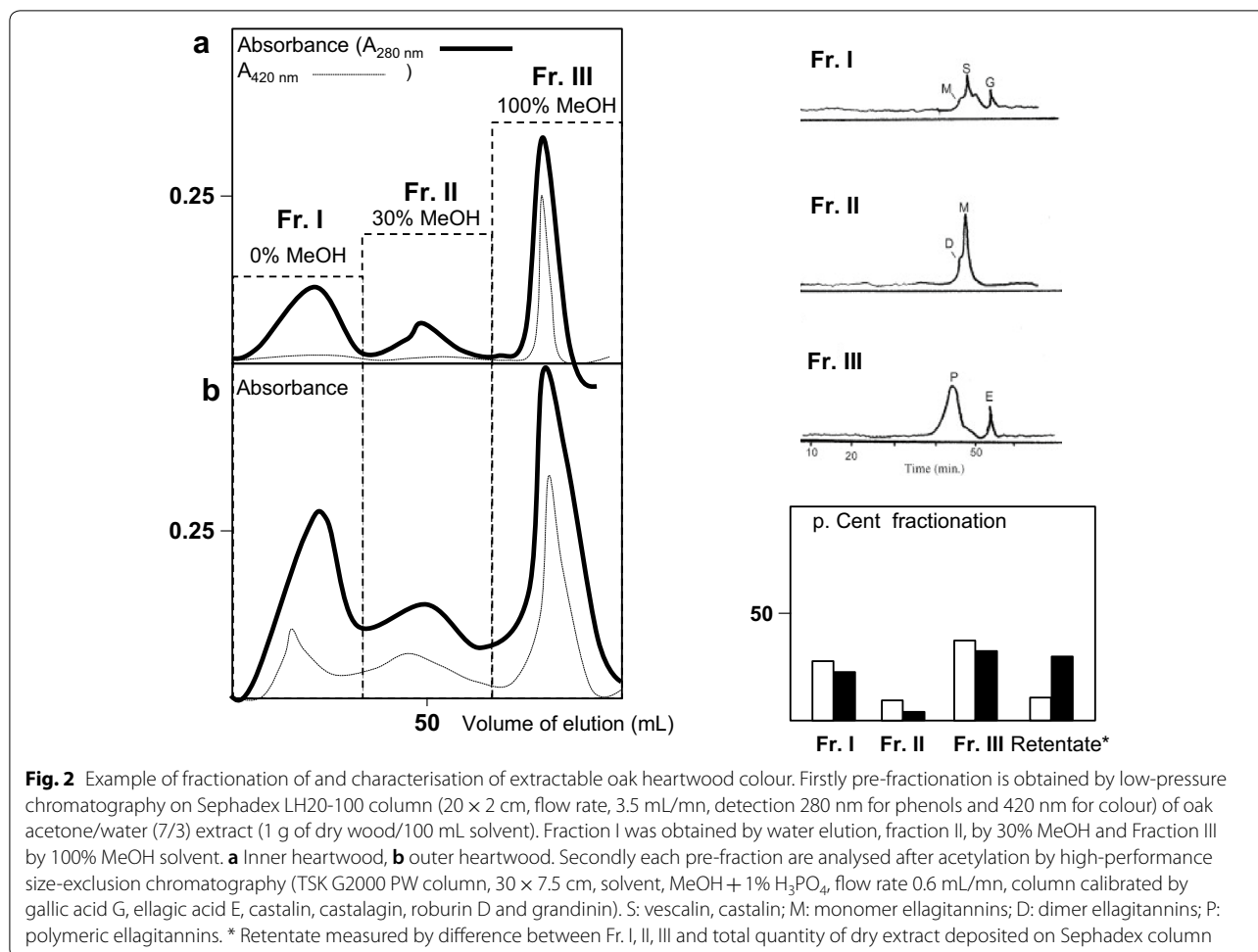
<sup>b</sup> CIE L\*, a\*, b\* measures and calculated parameters with a\*, b\* chromaticity coordinates C\* and h\*

red part of the colour more (a\*) than the yellow (b\*) and the lightness (L\*); the percentage of variation between the pure water and the ethanol/water (9/1) are 60% for a\*, 18% for b\* and 19% for L\* for *Q. robur* and 92%, 26% and 34% for *Q. petraea*.

For the extractable colour study, the acetone/water extraction of the heartwood sample presented a good compromise in terms of the quantities of dry extract, polyphenols and colouring compounds concentration.

**Fractionation and characterisation of extracted colour**

A study of the fraction obtained after a two-step sequential isolation permitted to characterise the composition of colour extracted from oak heartwood. In the first step, low-pressure chromatography on the Sephadex with a series of elutions (water, 30% MeOH, 100% MeOH) obtained 3 fractions. In the second step, the use of high-performance size-exclusion chromatography with TSKgel® G2000 in the solid phase allowed to obtain the precise composition in accordance with the weight of compounds in acetylated form. An example of this



procedure is shown in Fig. 2. The inner heartwood presented a lower quantity of products with the majority of fraction III eluted with pure MeOH containing the main part of the colouring material. For the outer heartwood, the extract was richer in fractions I and II, as opposed to the inner heartwood. The second step of fractionation permitted us to determine the composition of the fractions. Fraction I, with water elution, presented low weight phenols such as gallic acid, castalin and vescalin and a limited amount of monomer ellagitannins (castalagin and vescalagin). Fraction II, with a 30% MeOH elution, presented both monomer and dimer ellagitannins. Fraction III, with a 100% MeOH elution, presented low polarity phenols as ellagic acid and a polymeric form of ellagitannins. The third fraction contained a concentrate of the main part of the colouring compounds of the heartwood extract. The elution of the total extract deposited in the Sephadex column was never complete; the higher polymer, with strong brown colour, was retained on the first

2 cm of gel. The retentate represented 12.6% of the outer heartwood and 28.8% for inner heartwood.

During duraminisation, the ageing of the heartwood contributes to the decrease in the acetone/water extract due to the insolubilisation of a part of the ellagitannins polymers [12]. However, in proportion, the inner heartwood provided a smaller amount of the extract and a lower quantity of fraction III; however, it had a higher colouring intensity than the outer heartwood. Therefore, soluble oak heartwood colour was attributed to the polymeric form of ellagitannins.

**The nature of soluble colour and in vitro production via oxidative reactions**

In searching for an eventual link among colouring ellagitannins polymers, heartwood ageing and oxidation reaction, some specific reactions in vitro were studied. The influence of oxidation during duraminisation was followed by a fresh acetone/water extract of the outer heartwood of *Q. robur*, which is richer in

**Table 2** In vitro model of ellagitannins chemistry during duraminisation process: influence of oxidation (1 vs. 2)<sup>1</sup>; influence of association of oxidised ellagitannins and polysaccharides (a vs. b)<sup>2</sup> (initial extract from acetone/water (7/3, v/v) extraction of *Q. robur* outer heartwood)

	Dry extract colour		Chromatic values				
	%	O.D. <sub>420 nm</sub>	L*	a*	b*	C*	h*
1—Fresh extract							
Total	100	0.60	46.6	7.3	21.1	22.3	1.2
a—ellagitannins	70.4	0.25	37.1	6.3	17.3	18.4	1.2
b—EP complex	29.6	0.35	32.9	5.5	17.9	18.7	1.3
2—Oxidised extract <sup>1</sup>							
Total	100	0.82	39.1	13.7	34.6	37.2	1.2
a—ellagitannins	27.6	0.20	48.7	8.8	29.1	30.4	1.3
b—EP complex	72.4	0.62	25.1	3.7	9.9	10.5	1.2

<sup>1</sup> 30 days at 20 °C with air exposition in solution: 12% vol. EtOH, 5 g/L tartaric acid, pH 3.5, NaF, 100 mg/L

<sup>2</sup> a: soluble in EtOH/water 9/1, b: insoluble in EtOH/water 9/1

low weight and lightly coloured ellagitannins than the inner heartwood. After 30 days of oxidation, we compared the colour of the control solution to the oxidised solution (Table 2, experiments 1 and 2). During oxidation several reactions occurred; one of them concerns the formation of a copolymer with oxidised polymeric ellagitannins and polysaccharides (EP). Separation by selective precipitation is conducted before and after oxidation: the ellagitannins (soluble in EtOH/water, 9/1) and the ellagitannins–polysaccharides complex (insoluble in EtOH/water, 9/1). The results are shown in Table 2 (experiments a and b).

During oxidation, the proportion of the EP complex remained higher. In this experiment, 29.6% of the EP complex was in fresh extract, and 72.4% was present after oxidation. The quantity of ethanol in a 10 to 50% vol. solution had no effect on the final results; increasing the pH to 4.5 had similar results but earlier, after only 10 days. After oxidation, the yellow colour measured at OD 420 nm increased due to the EP complex contributions to the total colour. The lightness of the colour was lower for EP and higher for ellagitannins, probably due to the integration of dark polymeric ellagitannins in the EP complex during the oxidation reaction. The total extract after oxidation was redder and yellower in colour, signifying marked colour saturation ( $a^* + b^*$  higher).

The main part of the inner heartwood soluble colour was explained by oxidation involving polymerisation of the ellagitannins and a large association with polysaccharides. The colour formed was lower (light  $L$ ) with a mix of red (high  $a^*$ ) and yellow (high  $b^*$ ).

Pyrolysis/GC/MS of Fraction III at 500 °C produced furanoic derivatives typical of pyrolysis/GC/MS of polysaccharides and phenolics with various methoxy and

alkyl substituents typical of GC/MS of lignins (Fig. 3a) [19]. THM/GC/MS of Fraction II (30% MeOH) and Fraction III (100% MeOH) at 500 °C produced a pyrogram in which we identified same peaks 1, 2, 3, 4 and 5 typical of THM/GC/MS of ellagic acid [20]. Example for Fraction III is presented in Fig. 3b. Finally NMR spectra of Fraction III (<sup>1</sup>H, HSQC and HMBC) confirmed the presence of phenolic compounds (hexahydroxydiphenol, HHDP) and polysaccharides (Table 3) [21].

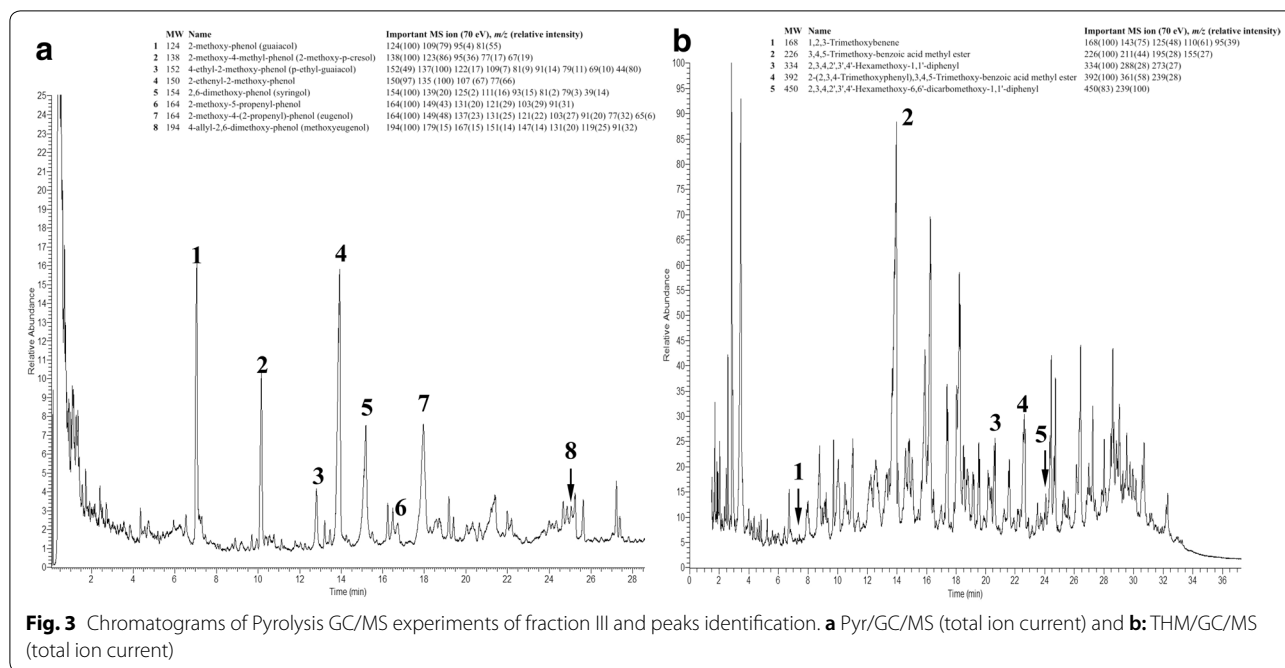
## Discussions

### Colour extraction and concentration

Oak heartwood has a natural yellow/brown colour, with a different intensity of lightness according to age and position in the trunk [14]. This property is not limited to oak wood [22]. Although colour is in relation to polyphenol extraction, especially ellagitannins [13], other parameters such as wood structure, anatomy and genetics may also affect it [10].

Solvents have a strong effect on the efficiency of colour extraction. Oak heartwood is rich in water-soluble extracts [13], but these extracts present heterogeneous composition with different groups of polymers: polysaccharides, ellagitannins and part of the low weight lignans [23]. Because the major origin of colour is from ellagitannins [14], solvent range and mixture on those that can be extracted quickly with a focus on ellagitannins, mainly polymeric ellagitannins and the corresponding part of soluble colour are specifically investigated. Ethanol and methanol with 10% vol. water were used to quickly obtain a good dry extract that was rich in polyphenols with dark yellow colour components. Acetone with 30% vol. water presented the best compromise with a higher dry extract, polyphenols index and colourful matter in accordance with previous investigations [11]. For polymeric





**Table 3** <sup>1</sup>H and <sup>13</sup>C (HSQC and HMBC) NMR (600 MHz, 298 K) assignments for Fraction III

<sup>1</sup> H NMR	<sup>13</sup> C NMR (HSQC and HMBC)	Groups and functions
0 < δ < 2.5	11 < δ < 57	Alkyl substituents
3 < δ < 5.5	53 < δ < 101	Sugar rings
6.3 < δ < 7.5	102 < δ < 168.2	Phenolics

ellagitannins and the corresponding colour fraction, water seems to be necessary to improve the extraction, probably due to wood fibres hydration [24, 25] and the improvement of polarity of the solvent mixture. Diethyl ether and MeOH–chloroform, both low polarity solvents, produced limited and lightly coloured dry extract containing low weight polyphenol.

Maintaining the temperature to 80 °C, as well as raising the pH to 8–10, improved the rate of extraction but also by intense degradation, yielded additional colouring matter not corresponding to those naturally present in heartwood (data not shown).

The reliability between the species of *Q. robur* and *Q. petraea* concerning colour parameters are uncertain, even if the species affected the ellagitannins concentration [10, 26] and the ellagitannins represent the main precursor of heartwood colour. At least a part of the difference can be attributed to the sampling procedure; the other part could be determined by genetics [10].

**Characteristics and evolution of oak heartwood colour**

During the heartwood ageing colour change, the colour becomes darker, redder and yellower, which increases with tree age [9]. Alongside the natural colour evolution, some fungal infections promote red–orange [27] and brown [28] discoloration, as do climatic accidents such as plain frost [29]. This type of wood must be excluded from investigations into the natural colour of oak heartwood.

By liquid extraction, such as with efficient solvents or a mixture of solvents, all heartwood colours are not completely removed from the wood [13]. The repartition of soluble colour from heartwood is not constant; the quantity increases during the ageing of the heartwood and explains why colour is more abundant in the inner heartwood than in the outer heartwood [14]. Colour progressively changes along the heartwood in relation to its age from light and a weak yellow to darker and brown for older wood in the middle of the tree. The water solubility of the extracts and the colour follow the same tendency [13]. A large part of the soluble heartwood colour, but not all, is due to the polymeric form of ellagitannins. Further sources of colour exist that are redder than polymerised ellagitannins, which are derived from specific lignans [30].

Similar reactions occurred in *Castanea sativa* heartwood and probably explain its colour [12]. Although both hydrolysable tannins in woods such as *Quercus* or *Castanea* and condensed tannins in woods such as *Juglans*, *Larix*, *Pinus* or *Pseudotsuga* have a different chemical structure of polyphenols, similar relationships have

been presented to illustrate the wood colouring process [17, 31, 32]. In *Quebracho* heartwood, the increase in the molecular weight of condensed tannins from the outer heartwood to the inner heartwood is clearly demonstrated [33]. Furthermore, these types of reactions concern not only tannins but also the general polymerisation of simple phenolic compounds in polymers of undefined structure that are highlighted in various conifer woods [34].

As expected, wood colour is correlated with the dry extract materials and polyphenols content [22, 31] as well as the insoluble ellagitannins [11]. Polymerisation during ageing is a frequent reaction to promote the colour of the wood. At the beginning of a reaction, the majority of formed polymers remain soluble because of their limited molecular mass. For older heartwood, since polymers continuously increase in size, the water-soluble fraction and the soluble colour decrease.

#### Origin of colour

The soluble colour of the heartwood extract is a result of the complex reactions of oxidation and the copolymerisation with cell-wall polymers as polysaccharides. This reaction is probably non-enzymatic. The pre-heated treatment of wood samples (60 °C, 10 h) before extraction, solubilisation and oxidation in a solution by air oxygen did not affect the oxidation and polymerisation of the ellagitannins and the formation of corresponding colour compounds (data not shown). These reactions, which are spontaneous in heartwood during the natural duraminisation process, can be mimicked in an in vitro solution [14]. The oxidation promoted robust structural alterations of ellagitannin structure [14]. Using IR spectroscopy, we observed a decrease in vibration bands 3500–3200  $\text{cm}^{-1}$  due to the association or linkage of the OH functions of ellagitannins, the appearance of a signal at 1715  $\text{cm}^{-1}$  was attributed to the C=C bonds, and a preponderance of signals occurred in the epoxy structures (1250  $\text{cm}^{-1}$ , 830–890  $\text{cm}^{-1}$ ). This unsaturated system allowed some complicated reactions with several non-tannin compounds as polysaccharides [35]. Additional research studies were conducted for investigating the nature of soluble coloured polyphenolics polymers found in old heartwood.  $^1\text{H}$  NMR spectra of polymers presented specific signals of hexahydroxydiphenol moiety of ellagitannins, as well as specific signals of polysaccharides. This was then confirmed by the main products identified in THM/GC/MS and pyrolysis/GC/MS experiments, respectively for ellagitannins and polysaccharides. Despite in lower quantity, the presence of substantial pyrolytic derivatives obtained from lignins highlighted the complexity of the heteropolymers structures. These different sources of structural information confirmed the

nature of polymers formed by the heterocondensation of various compounds, with a major part of ellagitannins and polysaccharides. This kind of products was well-reported in previous works [13, 33, 34].

Moreover, the ellagitannins are well-known highly oxidised compounds with consequential biological activity [36, 37]. Most chemical oxidation and UV light also impact wood colour through a photodegradation process [38]. However, this reaction is limited to the exposed surface and any global influences in heartwood colour appear weak.

#### Conclusion

Because colour presents a high interest for presentation and quality of aged spirits, investigation on colour origin are justified. In this target, a significant source of heartwood colour is elucidated.

Heartwood dry extract present a large part of colouring material and polyphenols. In this fraction, soluble part of ellagitannins, especially the polymeric forms are the main components for yellow–brown natural wooden colour. The proportion of coloured ellagitannins polymers fraction increase during trees heartwood ageing; due to oxidative reactions. Ethanol is a good solvent for these polymers, but water are necessary (10 to 30% vol.) for fibres hydration and improving the quantity of dry extract, polyphenols as ellagitannins and colour material.

For this consideration, the soluble polymeric ellagitannins possibly participated to a large part of spirit colour after a casks ageing process.

#### Abbreviations

CIE: Commission international de l'éclairage; MeOH: Methanol; EtOH: Ethanol; NaOH: Sodium hydroxide solution; EP: Ellagitannins–polysaccharides complexes; O.D.: Optical density; THF: Tetrahydrofuran; IR: Infrared.

#### Acknowledgements

SOGIBOIS splitting mills and Demptos cooperation for provided the wood samples. American Journal Experts for revision of the manuscript.

#### Authors' contributions

NV, MFNB and NVG designed the experiments and were major contributors in writing the manuscript; CM and CR performed the experiments; all authors contributed to interpretation of the results. All authors read and approved the manuscript.

#### Funding

This research was sponsored by the Demptos cooperation, member of TFF group (Tonnellerie François Frères) and by a grant for equipment's of Region Aquitaine.

#### Availability of data and materials

Not applicable to this article, because no datasets were generated or analysed during this study.

#### Competing interests

Authors declare that they have no competing interests.



Received: 11 March 2019 Accepted: 1 March 2020

Published online: 16 March 2020

## References

- Janin G, Mazet JF (1987) Mesure de la variabilité de la couleur du bois. Nouvelle méthode appliquée aux carottes de sondages. *Ann Sci For* 44:119–126
- Janin G, Mazet JF, Flot JL, Hofmann P (1990) Couleur et qualité du bois de chêne de tranchage: Chêne sessile, chêne pédonculé et chêne rouge. *Rev For Fr* 43:134–139
- Mazet JF, Janin G (1990) La qualité de l'aspect des placages de chênes: mesures de couleur et critères d'appréciation des professionnels français et italiens. *Ann Sci For* 47:255–268
- Nepveu G (1990) Les facteurs influençant la qualité du bois de chêne (chêne rouvre et chêne pédonculé). *Rev For Fr* 42:128–133
- Johnson H (1989) The story of wine. Mitchel Beazley, London, p 450
- Bamforth CW, Ward RE (2014) The Oxford handbook of food fermentations. Oxford University Press, Oxford, p 815
- Bauch J (1984) Discoloration in the wood of living and cut trees. *IAWA Bull* 5:92–98
- Haluk JP, Schoegel F, Metch M (1991) Chimie de la couleur du bois. Etude comparative des constituants polyphénoliques dans le chêne sain et le chêne coloré. *Holzforshung* 45:437–444
- Clumpers J, Janin G, Becker M, Levy G (1993) The influence of age, extractive content and soil water on wood color in oak: the possible genetic determination of wood color. *Ann Sci For* 50:403–409
- Mosedale JR, Charrier B, Janin G (1996) Genetic control of wood colour, density and heartwood ellagitannin concentration in European oak (*Quercus petraea* and *Q. robur*). *Forestry* 69:111–124
- Peng S, Scalbert A, Monties B (1991) Insoluble ellagitannins in *Castanea sativa* and *Quercus petraea* woods. *Phytochem* 30:775–778
- Viriot C, Scalbert A, Hervé du Penhoat CLM, Moutounet M (1994) Ellagitannins in woods of sessile oak and sweet chestnut dimerization and hydrolysis during wood ageing. *Phytochem* 36:1253–1260
- Clumpers J, Scalbert A, Janin G (1994) Ellagitannins in European oak wood: polymerization during wood ageing. *Phytochem* 36:1249–1252
- Vivas N, Nonier MF, Vivas de Gaulejac N, Pianet de Boissel I (2004) Occurrence and partial characterization of polymeric ellagitannins in *Quercus petraea* Liebl. and *Q. robur* L. wood. *Acad Sci CR Chimie* 7:945–954
- Vivas N, Laguerre M, Glories Y, Bourgeois G, Vitry C (1995) Structure simulation of two ellagitannins from *Quercus robur* L. *Phytochem* 39:1193–1199
- Vivas N, Glories Y, Bourgeois G, Pianet I, Vitry C, Barbe B (1996) Origine de la vescaline et de la castaline du bois de coeur de *Quercus petraea* Liebl. In: Vercauteren J, Cheze C, Dumon MC, Weber JF (eds) Polyphenols communication. Groupe polyphenols, Bordeaux pp, pp 41–42
- Scalbert A, Duval L, Peng S, Monties B, Herve du Penhoat CLM (1990) Polyphenols of *Quercus robur* L. II-Preparative isolation by low-pressure and high-pressure liquid chromatography of heartwood ellagitannins. *J Chromatogr* 502:107–119
- Vivas N, Vivas de Gaulejac N, Nonier MF (2003) Sur l'estimation et la quantification des composés phénoliques des vins. *Bulletin OIV* 865(866):281–303
- Nonier MF, Vivas N, Vivas de Gaulejac N, Absalon C, Soulié P, Fouquet E (2006) Pyrolysis-gaz chromatography/mass spectrometry of *Quercus* sp. Wood. Application to structural elucidation of macromolecules and aromatic profiles of different species. *J Anal Appl Pyrolysis* 75:181–193
- Galletti GC, Bocchini P (1995) Behaviour of catechin and ellagic acid subjected to thermally-assisted hydrolysis-methylation/gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 9:250–254
- Vivas N (1997) Recherches sur la qualité du chêne français de tonnellerie (*Q. petraea* Liebl., *Q. robur* L.) et sur les mécanismes d'oxydoréduction des vins rouges au cours de leur élevage en barriques. Ph D Bordeaux University, I:169p, II:52p. <https://www.theses.fr/1997BOR20504>
- Gierlinger N, Jacques D, Grabner M, Wimmer R, Shwanninger M, Rozenberg P, Pâques E (2004) Colour of larch heartwood and relationships to extractives and brown-rot decay resistance. *Trees* 18:102–108
- Nonier MF, Vivas N, Vivas de Gaulejac N, Absalon C, Vitry C, Fouquet E (2005) Global fractionation of heartwood extractable polymers (lignins, polysaccharides and ellagitannins) by selective precipitations. *J Sci Food Agric* 85:343–353
- Stone JE, Scallan M (1968) A structural model for cell wall water swollen pulp fibers based on their accessibility to macromolecule. *Cell Chem Technol* 2:343–358
- Rosen HN (1978) Interaction of alcohols and organics acids with wood. *Wood Sci* 10:151–157
- Nepveu G (1984) Contrôle héréditaire de la densité et de la rétractabilité du bois de trois espèces de chêne (*Quercus petraea*, *Quercus robur*, *Quercus rubra*). *Silvae Genet* 33:91–95
- Mosedale JR, Feuillat F, Baumes R, Dupouey JL, Puech JL (1998) Variability of wood extractives among *Quercus robur* and *Quercus petraea* trees from mixed stands and their relation to wood anatomy and leaf morphology. *Can J For Res* 28:994–1006
- Bauch J, Hundt HV, Weibman G, Lange W, Kubel H (1991) On the causes of yellow discolourations of oak heartwood (*Quercus* sect. *robur*) during drying. *Holzforshung* 45:79–85
- Charrier B, Janin G, Haluk JP, Mosedale JR (1995) Colour and chemical characteristics of moon rings in oakwood. *Holzforshung* 49:287–292
- Nonier MF, Vivas N, Vivas de Gaulejac N, Fouquet E (2009) Origin of brown discoloration in the staves of oak used in cooperage. Characterization of two new lignans in oak wood barrels. *Acad Sci CR Chimie* 12:291–296
- Burtin P, Jay-Allemand C, Charpentier JP, Janin G (1998) Natural wood colouring process in *Juglans* sp. (*J. nigra*, *J. regia* and hybrid *J. nigra* 23 x *J. regia*) depends on native phenolic compounds accumulated in the transition zone between sapwood and heartwood. *Trees* 12:258–264
- Dellus V, Scalbert A, Janin G (1997) Polyphenols and colour of Douglas fir heartwood. *Holzforshung* 51:291–295
- Roux DG, Evelyn SR (1960) Condensed tannins. 4. The distribution and deposition of tannins in the heartwoods of *Acacia mollissima* and *Schinopsis* spp. *Biochem J* 76:17–23
- Hegert HL (1992) Plant polyphenols: Biogenesis, chemical properties, significance. Hemingway R, Karchesy JJ (eds) Plenum press, New York, p 109
- Mc Manus JP, Davis KG, Beart JE, Gaffney SH, Lilley TH, Haslam E (1985) Polyphenols interactions. Part 1. Introduction; some observations on the reversible complexation of polyphenols with proteins and polysaccharides. *J Chem Soc Perkin Trans II*:1429–1438
- Yoshida T, Hatano T, Ito H, Okuda T (1999) Highly oxidized ellagitannins and their biological activity. In: Gross GG, Hemingway RW, Yoshida T, Branham SJ (eds) Plant polyphenols 2, vol 66. Springer, Boston, pp 127–144
- Barbehenn RV, Jones CP, Hagerman AE, Karonen M, Salminen JP (2006) Ellagitannins have greater oxidative activities than condensed tannins and galloyl glucoses at high pH: potential impact on caterpillars. *J Chem Ecol* 32:2253–2267
- Zahri S, Belloncle C, Charrier F, Pardon P, Quideau S, Charrier B (2007) UV light impact on ellagitannins and wood surface colour of European oak (*Quercus petraea* and *Quercus robur*). *Appl Surf Sci* 253:4985–4989

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.