Demonstration of the Presence of Carotenoids in Wood: Quantitative Study of Cooperage Oak

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The presence of β-carotene and a precursor of lutein is described in wood for the first time. These compounds were found in the wood of sessile oak, pedunculate oak, American white oak, chestnut, and beech. The levels were generally low and varied considerably from one tree to another. Among the European oaks, sessile oak was significantly richer in lutein than pedunculate oak. Sessile oaks from Jupilles forest contained the highest β-carotene levels. The position of the wood sample in the trunk affected the carotenoid content, and barrel wood is taken from a zone that is fairly rich in these compounds.

Keywords: *Carotenoids; oak wood; age of wood; European oak; American oak*

INTRODUCTION

Carotenoids are polyene hydrocarbons biosynthesized from eight isoprene units (tetraterpenes). These pigments are widespread in plant chloroplasts and certain microorganisms, where they play an important role in photosynthesis mechanisms (Calvin, 1955; Cholnoky *et al*., 1956). Indeed, these pigments enable plants to perform photosynthesis at wavelengths of between 380 and 550 nm which are not absorbed by chlorophyll. However, the presence of such compounds has been described in numerous kinds of nonphotosynthetic tissue such as roots and certain bacteria and in tissues in which photosynthesis activity is secondary, as in flowers and some fruits and vegetables (Goodwin, 1976). Carotenoids have been the subject of much scientific work in the food sector, in particular because these compounds are precursors of vitamin A. It is also known that carotenoids may lead to the formation of norisoprenoids, compounds that possess definite odor features through oxidative degradation catalyzed by light (Isoe *et al*., 1969) or by coupled oxidation in the presence of lipoxygenase (Belitz and Grosch, 1982). All these mechanisms were described by Crouzet and Seck (1982).

The grape berry carotenoids first demonstrated by Curl (1964) are thought to be the origin of the norisoprenoids found in grape (Schreier *et al.*, 1976), wine (Schreier *et al.*, 1980), and spirits distilled from wine (Simpson *et al.*, 1977; Masuda and Nishimura, 1980). Aging in oak barrels is also thought to be a source of enrichment of wines and wine spirits in norisoprenoids. Nishimura *et al.* (1983) and, more recently, Sefton *et al.* (1990) demonstrated that European and American cooperage oak contains norisoprenoids. Might carotenoids be the source of norisoprenoid formation as hypothesized in fruits and vegetables? If so, this would be a novel example of the presence of carotenoids in nonphotosynthetic tissues. This demonstration includes a quantitative study aimed at determining the distribution of these compounds in the trunk and comparing oak wood of different origins.

MATERIALS AND METHODS

Sampling. The samples of European oak were taken from 24 trees felled in France in the winters of 1991 and 1992. They consisted of six trees from the Limousin region (Dordogne), six from Tronçais forest (Allier), six from Jupilles forest (Sarthe), and six from Darney forest (Vosges). Leaves and acorns collected on the branches (Limousin, Jupilles, and Darney) or at the foot of the trees (Tronçais) were used to determine the botanical identify of the trees according to the morphological characters described by Dupouey and Badeau (1993). The six trees from the Limousin region were pedunculate oaks (*Quercus robur* L.), and the 18 trees from Tronçais, Jupilles, and Darney forests were sessile oaks (*Quercus petraea* Liebl.). The pedunculate oaks were 73−116 years old, and the sessile oaks were 144−229 years old. A disk of wood 15 cm thick was cut 1.50 m from the base of the trunk of the pedunculate oaks and 3.50 m from those of the sessile oaks. The splitter marked the heartwood with the positions of the four "virtual" staves corresponding to the four cardinal points (N, S, E, and W). Shavings were planed in the zones marked by the splitter. These shavings were ground to a particle size of less than 0.5 mm and kept at −20 °C before use. The trees were compared using a homogeneous mixture of wood powder from the four directions.

The American white oaks used, identified as *Quercus alba* L. using the morphological characteristics described by Cooper and Watt (1973), were from the states of Missouri, Pennsylvania, and Virginia. The extremities of three staves of each provenance were planed. The sample of each of these origins consisted of a homogeneous mixture of wood powder from the three staves. The samples of chestnut wood (*Castanea sativa* Mill.) and beech wood (*Fagus silvatica* L.) were from trees felled in Lozère (France).

A disk cut 1.50 m from the ground in the trunk of a sessile oak (Tronçais no. 1) was also used for study of the carotenoid content of the wood according to its age. Eight wood fractions of increasing age were planed from the sapwood (A) to the center of the trunk (F7) (A, 0−17 years; F1, 17−40 years; F2, 40−60 years; F3, 60−80 years; F4, 80−100 years; F5, 100−120 years; F6, 120−140 years; F7, 140−163 years).

Extraction of Carotenoids from Wood. The techniques used for carotenoid extraction and assay methods were based

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Table 1. Chromatographic and Spectroscopic Properties of the Carotenoids Identified in Oak Wood

carotenoids	t_R[a]	R_f[b]	spectral data, λ_{max} (nm) HPLC eluent	spectral data, λ_{max} (nm) petroleum ether	ethanol	hypsocromic shift in ethanol (nm)[c]	acetylation
neoxanthin	3.5		415, 438, 468				
violaxanthin	5.2		415, 441, 471				
lutein	9.6	0.41	(427), 450, 478	422, 446, 474	422, 445, 476	0	+
β-carotene	19.1	0.94	(425), 454, 480	(425), 448, 475	(427), 450, 476	0	−

[a] HPLC conditions: C18 5 μm column; gradient of 0−20 min acetone:water (80:20) to 100% acetone, 20−30 min 100% acetone. [b] TLC support: silica gel; solvents, petroleum ether:acetone:diethylamine (10:4:1). [c] On addition of hydrochloric acid.

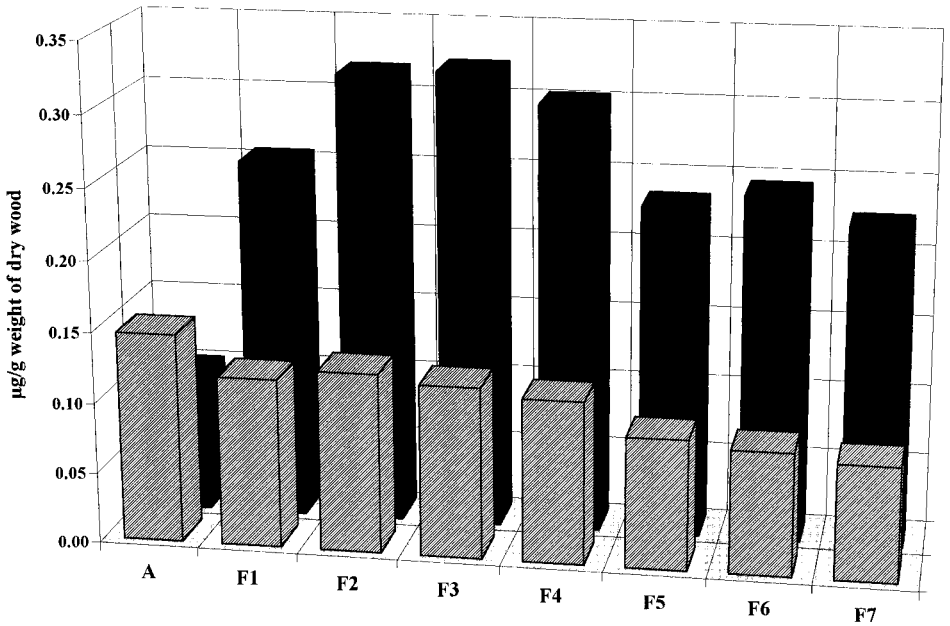

Figure 1. Lutein (black) and β-carotene (gray) contents in μg/g of dry weight of wood in eight wood fractions of increasing age taken from the sapwood (A) to the center (F7) of a sessile oak from Tronçais forest.

on those developed by Razungles (1987) for grape berries. Fresh wood powder (10 g) was mixed with magnesium carbonate (3 g) to neutralize the free acids in the wood that might cause isomerization reactions in the carotenoids. A known quantity of internal standard, β-apo-8′-carotenal (Fluka, Bucks, Switzerland), was placed in liquid form on this mixture. Carotenoids were extracted from the wood by 6 × 30 mL of redistilled acetone. The acetone extracts were separated from the wood by filtration on a No. 4 sintered glass filter and evaporated to dryness in a Rotavapor. The residue was added to an ethanol:potassium hydroxide mixture (50 mL of ethanol + 5 mL of 60% KOH in water). The saponification stage lasted for 12 h at 4 °C; its purpose was to eliminate lipids and to precipitate polyphenols in the alcohol phase. The saponification mixture was then placed in a separating funnel with 50 mL of deperoxidized ethyl ether. The ether was washed with water, dried on NaCl, and evaporated to dryness. The residue was resuspended in 2 mL of acetone. The final acetone extract containing wood carotenoids was filtered before HPLC injection. All these operations were performed in the shade with no direct light in order to prevent the photodegradation of carotenoids.

HPLC Assay of Carotenoids. Analysis of carotenoids was performed using a set of Waters (Millipore) HPLC apparatus consisting of the following items: a 490 E multiwave length detector, two 510 pumps, an automatic injector, and an SIM (system interface module). A Gilson 231 automatic injector was used. The Maxima 820 program (Waters, Millipore) was loaded in a Powermate 386/25 (Nec) microcomputer. A binary gradient was used. Solvent A was acetone/water (80:20). Solvent B was acetone. Flow was 1 mL/min with a gradient of 0−100% B in 20 min, 100% B for 2 min, 100−0% B in 1 min, and 12 min of re-equilibration under the initial conditions. A Nucleosil 5-C 18 column (250 mm × 4 mm, 5 μm; Merck) was used. Study of the UV−visible spectra of the carotenoid compounds was performed using a Waters 990 diode array detector. Carotenoid assay was performed at 450 nm. The response factors of the compounds studied were established using a range of standard solutions: β-carotene and lutein were purchased respectively from Merck, Darmstadt, Germany, and Extrasynthèse, Genay, France; violaxanthin and neoxanthin were isolated from nettles as previously described (Razungles et al., 1996). A homogeneous batch of wood powder was divided into five equal parts that were extracted, saponified, and analyzed by HPLC. The repeatability of the method according to the coefficient of variation of five results was 8% for lutein and 10% for β-carotene.

TLC Assay of Carotenoids. In order to confirm the identification of carotenoids, the extracts were separated by TLC (silica gel G60 plates, thickness 0.7 mm) using petroleum ether−acetone−diethylamine (10:4:1) (Minguez-Mosquera *et al.*, 1993).

RESULTS AND DISCUSSION

Identification of Carotenoids in Wood. The final acetone extract of a sample of oak wood was subjected to TLC. Lutein and β-carotene were separated and recovered for individual analysis (Minguez-Mosquera *et al.*, 1993). Identifications were confirmed by comparison with reference substances in different solvents using R_f values and the absorption peaks of the UV−visible spectra and by a chemical test: acetylation for hydroxyl groups (Davies, 1976) (Table 1).

It was demonstrated for the first time, to the best of our knowledge, that two carotenoids, β-carotene and a lutein derivative, are found in oak wood. This is additional evidence for the presence of carotenoids in nonphotosynthetic plant tissue (Goodwin, 1976). These compounds could result in the presence of β-ionone (Nishimura *et al.*, 1983) and some of the 30 other norisoprenoid substances (Sefton *et al.*, 1990) identified in oak wood.

Table 2. Lutein (Lut), β-Carotene (β-car), and Lutein + β-Carotene (Lut + β-car) Contents (μg/g Dry Weight of Wood) and Lutein:β-Carotene (Lut:β-car) Ratio[a]

species	forest	tree	lut	β-car	Lut + β-car	Lut:β-car
pedunculate oat	Limousin	L1	0.24	0.15	0.39	1.62
		L2	0.11	0.10	0.22	1.12
		L3	0.22	0.22	0.44	1.01
		L4	0.20	0.14	0.33	1.45
		L5	0.13	0.18	0.31	0.73
		L6	0.19	0.56	0.76	0.35
		L = P	0.18	0.22	0.41	1.04
		CV	27.3	75.8	45.9	44.6
sessile oak	Tronçais	T1	0.31	0.06	0.37	5.25
		T2	0.59	0.28	0.87	2.09
		T3	0.09	0.07	0.16	1.25
		T4	0.52	0.46	0.98	1.12
		T5	0.51	0.45	0.97	1.13
		T6	0.40	0.43	0.83	0.94
		T	0.40	0.29	0.70	1.96
		CV	45.2	64.3	49.6	84.5
	Jupilles	J1	0.39	0.41	0.80	0.94
		J2	0.50	0.28	0.78	1.80
		J3	0.23	0.58	0.81	0.39
		J4	0.70	0.66	1.36	1.07
		J5	0.38	0.69	1.07	0.55
		J6	0.55	0.39	0.94	1.40
		J	0.46	0.50	0.96	1.02
		CV	35.6	32.8	23.2	51.3
	Darney	D1	0.36	0.14	0.49	2.64
		D2	0.26	0.19	0.44	1.37
		D3	0.20	0.16	0.36	1.30
		D4	0.37	0.17	0.54	2.17
		D5	0.22	0.06	0.28	3.81
		D6	0.22	0.24	0.46	0.94
		D	0.27	0.16	0.43	2.04
		CV	26.9	37.4	21.8	52.4
	Missouri	R	0.38	0.32	0.69	1.68
		CV	42.3	63.5	45.9	71.9
American white oak	Pensylvannia	M1	0.13	0.90	1.04	0.15
	Virginia	Pe1	0.20	0.59	0.79	0.35
		V1	0.12	0.39	0.52	0.32
		B	0.15	0.63	0.78	0.27
		CV	23.7	33.3	27.2	32.6
chestnut	Lozère	C1	0.21	0.66	0.87	0.33
beech	Lozère	H1	0.13	0.11	0.24	1.21

[a] The data are for wood of 6 pedunculate oaks (P) from the Limousin region (L), 18 sessile oaks (R) from Tronçais (T), Jupilles (J), and Darney (D) forests, 3 American white oaks (B) from the states of Missouri (M), Pensylvannia (Pe), and Virginia (V), and 1 chestnut (C) and 1 beech (H) from the Lozère department (France). CV, coefficient of variation, %.

Two hypotheses are envisaged to account for the presence of carotenoids in wood. They could come from leaves, in which they are synthesized in large quantities. However, carotenoids are hydrophobic and not soluble in elaborate sap. They may nevertheless be translocated as bound compounds which are more hydrophilic than free carotenoids. The second hypothesis is that of the *in situ* formation of carotenoids in the living cells in the sapwood assuming a protective role against singlet oxygen as reviewed by Larson (1988). Carotenoids would then be found subsequently in heartwood, as there is little diffusion of oxygen in heartwood and they are protected from oxygen by large quantities of highly oxidizable polyphenols.

Two other compounds in the carotenoid fraction present in distinctly smaller amounts were also studied. Their UV−visible absorption spectra in the chromatography solvent measured using the diode array detector were identical to those of violaxanthin and neoxanthin (Razungles *et al.*, 1996). Their retention times also corresponded to those of the two substances isolated from nettles. The compounds were present in tiny quantities on the thin layer and were not very visible; it was impossible to calculate their R_f or to recover them. Thus, these compounds were only tentatively identified. They are probably present in the wood only in trace amounts, and no quantitative study was undertaken.

Table 3. Results of Analysis of Variance Concerning the Carotenoid Content of the Wood of Pedunculate Oaks (P) from the Limousin Region (L) and Sessile Oaks (R) from Tronçais (T), Jupilles (J), and Darney (D) Forests

	effect[a]		classification in homogeneous groups[b]	
	species $F_{1,22}$	forest $F_{2,15}$	species	forest
lutein	**	NS	$\overline{\text{R > P}}$	
β-carotene	NS	**		$\overline{\text{J > T > D}}$
lutein + β-carotene	NS	**		$\overline{\text{J > T}} > \overline{\text{T > D}}$
lutein: β-carotene	NS	NS		

[a] Significant effects at thresholds of 5% (*), 1% (**), or 0.1% (***) or not significant (NS). [b] Species and forests are classified in descending order; the groups not significantly different are grouped under the same line.

Search for the Lutein Derivative. Preparation of the carotenoid extract included a saponification stage aimed at removing polyphenols and lipids from the wood. However, saponification breaks ester bonds and may release into the medium carotenoids that are in a bound form in the wood. Analysis of a non saponified extract confirmed the presence of β-carotene in free form in the wood. Lutein was absent from the chromatogram, but a new compound was present at the beginning of the analysis. Lutein is therefore not present in a free form in oak wood but as a bound form; the combination is eluted at the beginning of the chromatogram. The tentatively identified violaxanthin and neoxanthin were also present as derivatives in this wood.

The precursor of lutein was eluted earlier than lutein alone under the HPLC conditions used. This bound compound is therefore more polar than lutein alone. In addition, the UV−visible spectrum of the precursor shows maximum absorption at 325 nm. These two observations support the theory of a lutein−acid phenol combination.

An attempt was made to isolate the lutein precursor compound in order to confirm this. Attempts at fractionating raw extract using column and thin-layer preparative chromatography were unsuccessful. The amounts of precursor collected were far too small for use for identification using spectral methods. In addition, polyphenols were abundant in the raw extract, hindering the isolation of the compound.

Effect of the Age of Oak Wood on Its Carotenoid Content. Carotenoids were assayed in each of the eight wood fractions of increasing age taken from the sapwood (the youngest wood) to the heartwood (the oldest wood). The sapwood was richer in β-carotene than lutein because the ratio became reversed in the heartwood (Figure 1). The evolution of the lutein content during the aging of the wood is in two stages. In the first four fractions of the wood, the lutein levels increased with the age of the wood, especially during the transition from fraction A to fraction F1, corresponding to the transition from sapwood to heartwood. Lutein may also be partially eliminated from the sapwood by migration via ligneous ray cells and accumulate in the peripheral parts of the heartwood. The compound migrates better than β-carotene because it is bound to a hydrophilic compound. The decrease in the lutein content in the last five wood fractions can be accounted for by the slow

degradation of the compound. The highest β-carotene content was recorded in the sapwood; the level then fell steadily in the heartwood. The degradation of β-carotene and lutein probably results from identical reactions. Barrel wood is cut from fractions F1−F5, which are rich in carotenoids.

Incidence of the Botanical and Geographical Origins on the Carotenoid Content of the Wood. The results of assays of lutein and β-carotene on the extracts of wood of 6 pedunculate oaks, 18 sessile oaks, 3 American white oaks, 1 chestnut, and 1 beech are shown in Table 2. The extracts of wood of all five deciduous species studied contained carotenoids. In addition, lutein and β-carotene were found in the three oak species, regardless of the geographical origin of the trees. The lutein and β-carotene levels were nevertheless very low and in all cases less than 1.5 μg/g of dry wood. The values recorded ranged from 0.16 (T3) to 1.36 (J4) μg/g of dry wood. The coefficient of variation also showed substantial intertree variation in the lutein:β-carotene ratio. This varied from 0.15 (M1) to 5.25 (T1).

As the number of samples of American white oak, chestnut, and beech was not considered to be representative, only the data concerning European oak (pedunculate oak and sessile oak) were subjected to analysis of variance of each parameter (Lut, β-car, Lut:β-car, and Lut + β-car). Study of the species factor was performed using the 6 pedunculate oaks and the 18 sessile oaks. Study of the forest factor was performed for three forests of sessile oaks. These two factors were tested using the variance of the individual trees. The results of the analysis of variance are provided in Table 3. Lutein was more discriminant than β-carotene. The species effect was significant at the 1% threshold for lutein and not significant for β-carotene, the sum of the carotenoids and the lutein:β-carotene ratio. Sessile oak wood contained, on average, twice as much lutein as pedunculate oak wood. The forest effect was significant at 1% for β-carotene and for the sum of carotenoids. Among the sessile oaks, the trees from Jupilles forest were particularly rich in carotenoids. Analysis of variance between forests did not reveal a significant effect of the lutein content and the lutein:β-carotene ratio.

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LITERATURE CITED

Belitz, H. D.; Grosch, W. *Lehrbuch der lebensmittelchemie*; Springer-Verlag: Berlin, Heidelberg, New York, 1982; Vol. 788, pp 174−176.

Calvin, M. Function of carotenoids in photosynthesis. *Nature* **1955**, *176*, 1211−1215.

Cholnoky, L.; Gyorgyfy, C.; Elisabeth, N.; Martha, P. Function of carotenoids in chlorophyll-containing organs. *Nature* **1956**, *178*, 410−411.

Cooper, G. A.; Watt, R. F. Oak, an American wood. *US Gov. Agric.* **1973**, 1−9.

Crouzet, J.; Seck, S. L'arôme de la tomate, mécanismes de formation des constituants par voies biochimiques et chimiques. (Tomato aroma—mechanisms of formation of the constituents by biochemical and chemical means.) *Parfums, Cosmét., Arômes* **1982**, 44, 71−84.

Curl, A. L. The carotenoids of several low-carotenoids fruits. *J. Food Sci.* **1964**, *24*, 241−245.

Dupouey, J.-L.; Badeau, V. Morphological variability of oaks (*Quercus robur* L., *Quercus petraea* (Matt) Liebl., *Quercus pubescens* Willd) in northeastern France: preliminary results. *Ann. Sci. For.* **1993**, *50*, 35s−40s.

Goodwin, T. W. Distribution of carotenoids In *Chemistry and biochemistry of plant pigments*; Goodwin, Ed.; Academic Press: London, New York, 1976; Vol. I, 870 pp.

Isoe, S.; Hyeon, S. B.; Sakan, T. Photo-oxygenation of carotenoids. I. The formation of dihydroactinidiolide and β-ionone from β-carotene. *Tetrahedron Lett.* **1969**, *4*, 279−281.

Larson, R. A. The antioxidants of higher plants. *Phytochemistry* **1988**, *27*, 969−978.

Masuda, M.; Nishimura, K. Occurrence and formation of damascenone, trans-2,6,6-trimethyl-1-crotonyl-cyclohexa-1,3-diene, alcoholic beverages. *J. Food Sci.* **1980**, *45*, 396−397.

Minguez-Mosquera, M.; Hornero-Mendez, D. Separation and quantification of the carotenoid pigments in red peppers (*Capsicum annuum* L.), paprika, and oleoresin by reverse-phase HPLC. *J. Agric. Food Chem.* **1993**, *10*, 1616−1620.

Nishimura, K.; Ohnishi, M.; Masuda, M.; Koga, K.; Matsuyama, R. Reactions of wood components during maturation. In *Flavour of distilled beverages*; Pigott, J. R., Ed.; Ellis Horwood: Chichester, England, 1983; 241 pp.

Razungles, A.; Bayonove, C. L.; Cordonnier, R. E.; Baumes, R. L. Etude des caroténoides du raisin à maturité. (Carotenoids of mature grapes.) *Vitis* **1987**, *26*, 183−191.

Razungles, A. J.; Babic, I.; Sapis, J. C.; Bayonove, C. L. Particular behavior of epoxy xanthophylls during veraison and maturation of grape. *J. Agric. Food Chem.* **1996**, *44*, 3821−3825.

Schreier, P.; Drawert, F.; Junker, A. Identification of volatile constituents from grapes. *J. Agric. Food Chem.* **1976**, *24*, 331−335.

Schreier, P.; Drawert, F.; Abraham, K. O. Identification and determination of volatile constituents in burgundy Pinot noir wines. *Lebensm.-Wiss. Technol.* **1980**, *13*, 318−321.

Sefton, M.; Francis, I.; Williams, P. Volatile norisoprenoid compound as constituents of oak woods used in wine and spirit maturation. *J. Agric. Food Chem.* **1990**, *38*, 2045−2049.

Simpson, R. F.; Strauss, C. R.; Williams, P. J. Vitispirane: a C13 spiro-ether in the aroma volatiles of grape juice, wines and distilled grape spirits. *Chem. Ind.* **1977**, *6*, 663−664.

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