Wood Science and Technology 33 (1999) 271–283 © Springer-Verlag 1999

Waxes composition of *Quercus suber* reproduction cork from different Spanish provenances

E. Conde, M. C. García-Vallejo, E. Cadahía

Summary The chemical composition of waxes was studied in *Quercus suber* cork planks collected in seven localities of the three main production areas of Spain. Waxes were extracted with chloroform ($CHCl_3$) and the extract was submitted to saponification in order to obtain the neutral and acid fractions. The trimethylsilyl derivatives of both fractions were analysed by GC-MS.

The seven populations cannot be distinguished by their total contents of waxes (Chloroform extract) and of the neutral and acid fraction. This lack of differences could be due to the important variability among samples of each population.

The neutral fraction was mainly composed of fatty alcohols (all the even members from C_{18} to C_{26}, with traces of the intermediate odd members and some unsaturated groups) and triterpenes (11 components, among them friedelin, betulin and cerin were identified) and a very small amount of monocarboxylic fatty acids (C_{16} and C_{24} members) was also present. The main group of components of the acid fraction was that of fatty acids (saturated even C_{14}–C_{24} and odd C_{15}, C_{17}, C_{21} members, accompanied by a great amount of unsaturated terms and some ω-hydroxyacids, 18-hydroxy-9,12-octadecadienoic and 18-hydroxy-9-octadecenoic acids). Four triterpenes and the C_{20} and C_{24} alcohols were also detected in the acid fraction. Significant differences were found among the corks of the various provenances in the global contents of alcohols and triterpenes and in these individual components contents of the neutral fraction. Concerning the differences among the various provenances in the contents of the groups of fatty acids, alcohols, and triterpenes in the acid fraction, a higher number of differences were obtained in the comparisons of the contents of acids and triterpenes, whereas the differences were practically null in the case of the alcohols. When the differences were studied considering the individual compounds, the relation with

---

Received 12 December 1997

E. Conde, M.C. García-Vallejo (✉), E. Cadahía
INIA-CIFOR
Area de Industrias Forestales
Apdo. 8111
28080 Madrid, Spain

The authors are grateful to Dr. J. R. González Adrados for providing the cork samples and for his assistance on statistical analysis, to Mr. A. Sánchez for his help throughout the chemical analysis and to Ms. M. L. Cáceres for the sample preparation. This work was financially supported by the SC94-113 Project from M.A.P.A (Ministry of Agriculture, Fisheries and Food).

the behaviour of the contents of the groups of components was not as clear as it happens in the neutral fraction. In this case, the differences were scarcer and they did not show a unique pattern, neither in the components, nor the populations. There is no correspondence in the differences among the provenances regarding the components of the neutral fraction or those of the acid fraction. No clear relationship was found between the geographical proximity of the provenances and chemical similarity of the waxes composition.

Introduction

The studies of cork extraction with apolar solvents revealed the presence of some waxy materials, mainly consisting of fatty acids (Graça and Pereira, 1994; Conde et al., 1997b), aliphatic hydrocarbons (n-alkanes from C_{16}–C_{34}) and alcohols (all members with even numbers from C_{20}–C_{26}) (Bescansa López and Ribas Marqués, 1966; Bescansa López et al., 1966, Conde et al., 1999), as well as triterpenes from the families of friedelane and lupane, mainly cerin and friedelin (Pereira, 1979; Caldas et al., 1985; Conde et al., 1999).

In a previous work (Conde et al., 1999), we began the study of the changes in the chemical composition of the waxes contained in reproduction cork from Spanish *Quercus suber* throughout the industrial processing. Our results indicated that waxes presented an important variability as happens with other cork components, and the more pronounced changes in the experiments were observed after boiling, when the industrial processing is concluded with a resting period in the open air or inside a store-room.

In this paper, we describe the extraction and GC-MS analysis of the different groups of waxes components (fatty acids, fatty alcohols and triterpenes) and study the variability of these compounds in cork planks from several trees grown in seven different Spanish localities.

Material and methods

Samples: Reproduction cork samples were collected from trees grown in seven different localities of the three most important production areas in Spain: Andalucía, Extremadura and Cataluña. Table 1 includes these populations and their UTM coordinates. 3–5 trees were selected in each locality and pieces of the planks of each tree were chosen in order to obtain similar commercial quality cork samples.

The cork samples free of outer corkbark were ground and sieved. The powdered cork (2 g, 0.5–1 mm particle size) was exhaustively extracted in Soxhlet with $CHCl_3$ (8 h).

Table 1. Sample provenances and their UTM coordinates

Region	Province	Locality(∗)	Coordinates
Andalucía	Sevilla	Almadén de la Plata(1)	29SOC60192
Andalucía	Cádiz	Los Barrios(2)	30STF70822
Andalucía	Cádiz	Medina Sidonia(3)	30STF58722
Extremadura	Cáceres	El Chaparral(4)	29SPC97850
Extremadura	Badajoz	Jerez de los Caballeros(5)	29SPC85748
Cataluña	Gerona	Maçanet de Cabranys(6)	31TDG79592
Cataluña	Gerona	Forallac(7)	31TEG05339

(∗) Identification number in the text

Evaluation and saponification of cork waxes: $CHCl_3$ extract was dried under vacuum. Waxes contents were determined from the weight of $CHCl_3$ extract residue. The dried $CHCl_3$ extract was submitted to saponification with 50 ml 0.5 N KOH in EtOH, by boiling the mixture for 1 h under reflux. After addition of 50 ml H_2O, the saponification mixture was extracted with petroleum ether (3 × 15 ml). The petroleum ether fraction (neutral or unsaponified components fraction) was dried over anh. Na_2SO_4 and the solvent was distilled off under vacuum.

The remaining aqueous solution was acidified with 12 M HCl and extracted again with petroleum ether (3 × 15 ml). This second petroleum ether fraction, mainly composed of acid wax components, was also dried over anh. Na_2SO_4 and the solvent was distilled off.

Before their GC/MS analysis, the dried petroleum ether fractions were submitted to trimethylsilylation in order to convert the acids and hydroxyl groups of the waxes components into their more volatile TMS derivatives. This derivatization was carried out with N,O-bis-trimethylsilylacetamide (BSA) and trimethylchlorosilane as catalisator in pyridine.

GC/MS conditions: A HP 5890 gas chromatograph, equipped with a HP 5971 selective mass detector, and a HP-1 capillary column (12 m × 0.20 mm, film thickness 0.33 μm) was used with the following work conditions: column temperature program, 75 °C-325 °C (10 °C/min) and then isothermal at 325 °C for 10 min; injector and detector temperatures were 300 °C and 335 °C, respectively; carrier gas, He (0.5 ml/min), and ionisation energy, 70 eV.

The component identifications were carried out by direct comparison of their chromatographic retention times and mass spectra with those of standards and/or reported in literature. The quantitative evaluation of each component was performed by GC, and expressed as area percentage of the corresponding chromatographic peak referring to the total chromatogram area.

Statistical Analysis: Data were analysed using the BMDP package. Univariate analysis (BMDP P7D) and stepwise discriminant analysis (BMDP P7M) were carried out. In the univariate analysis, average, standard deviation and coefficient of variation were calculated, using a single-variable model. The pairwise *T*-test was also carried out in order to determine the significance levels of the differences of all the variables grouped by provenances. In the stepwise discriminant analysis, the variables used in computing the linear classification functions were chosen in stepwise manner (Jennrich and Sampson, 1985). Both forward and backward selection of variables was possible; at each step, the variable that added most of the separation of the groups was entered into (or the variable that adds the least is removed from) the discriminant function. The graphical representation of the projections of the points on the two principal canonical axes indicates the statistical distances among the groups.

Results and discussion

Table 2 shows the results of the chemical composition of waxes, expressed as percentages of chloroform ($CHCl_3$) extractives and neutral and acid fractions, indicating the average value of each variable for all populations. The comparison of the reported data of waxes contents with our results allows to conclude that the global average value (7.87%) of our results can be included in the range described in the literature (3.5–7.9%, Guillemonat 1960; Marcos de Lanuza, 1965; Carvalho,

Table 2. Chemical composition of waxes (% related to dry cork) of *Quercus suber* reproduction cork from different Spanish provenances

	1		2		3		4		5		6		7		Global э	
	x	CV	x	CV	x	CV	x	CV	x	CV	x	CV	x	CV	x	CV
$CHCl_3$ extractives	6.45	14	7.69	14	11.05	44	6.51	13	7.70	28	9.49	37	7.07	22	7.87	31
Neutral fraction	4.50	50	4.77	58	8.57	56	7.11	65	3.59	36	5.08	31	6.48	28	5.80	53
Acid fraction	0.90	24	1.02	79	2.02	81	1.49	19	1.38	45	2.94	30	2.41	19	1.71	46

1. Almadén de la Plata; 2. Los Barrios; 3. Medina Sidonia; 4. El Chaparral; 5. Jerez de los Caballeros; 6. Maçanet de Cabranys; 7. Forallac. Average (x) and coefficient of variation (CV) were calculated for 5 samples in Almadén de la Plata, Los Barrios, El Chaparral and Forallac, 4 samples in Medina Sidonia and Maçanet de Cabranys and 3 samples in Jerez de los Caballeros. э Average and CV were calculated for the whole 31 samples

1968; Hata et al., 1969; Holloway, 1972; Pes and Lissia, 1972; Pes, 1974; Pereira, 1988; Graça and Pereira, 1995).

The results of the application of the pairwise *T*-test to the waxes contents ($CHCl_3$ extractives) and the neutral and acid fractions, grouped by provenances, indicated that these variables presented scarce significant differences among the seven populations. Thus, chloroform extract and waxes neutral fraction did not show significant differences among the provenances. The only significant differences found were those of the acid fraction among the populations 1 *versus* 7 (at 5% level of significance). This lack of differences could be due to the important variability among samples of each population, very marked for the acid fraction in 2 and 3 and the neutral fraction in 1, 2, 3 and 4. This between-tree variability is in agreement with previous studies about cork composition (Pes and Lissia, 1972; Pereira, 1988; Cadahía et al., 1996; Conde et al., 1997; García-Vallejo et al., 1997). In fact, the studies of Marcos de Lanuza (1968) revealed the influence of the North or South orientation of the sample in the tree, the age of the tree, the distance of the sample from the base of the tree and the location of the sample in the stem or in the branch on the extracts contents of cork.

The results of the GC-MS analyses of both fractions of the chloroform extract from cork samples of different Spanish provenances are gathered in Tables 3 and 4, where the components are arranged according to their position in the GC-MS chromatogram. After waxes saponification with alcoholic KOH, the samples yielded complex mixtures of several types of aliphatic components: triterpenes, fatty acids (alkanoic and ω-hydroxy-alkanoic acids), and fatty alcohols (1-alkanols). As in our previous study on cork samples in different stages of the industrial processing (Conde et al., 1999), no n-alkanes were found in these extracts, in disagreement with the results of Bescansa López and Ribas Marqués (1966), who described the presence of a 0.6% of alkanoids in cork.

Table 3 includes the results of the composition of the waxes neutral fraction, for cork samples classified according to the different provenances. This fraction was mainly composed by fatty alcohols and triterpenes, and only a scarce presence of fatty acids. The fatty alcohols group (global average value 43.12%) was analysed as their trimethylsilyl ether derivatives and comprised all the even members from C_{18} to C_{26}, with traces of the intermediate odd members and some unsaturated terms that also appeared in the cork samples of the industrial pro-

Table 3. GC-MS analysis of neutral fraction of waxes of *Quercus suber* reproduction cork from different Spanish provenances. Concentrations are expressed as area % of the total derivative peak

		1		2		3		4		5		6		7		Total ϶	
		x	CV	x	CV	x	CV	x	CV	x	CV	x	CV	x	CV	x	CV
1	Octadecanol	0.39	107	1.14	40	2.49	77	0.39	98	0.60	18	1.57	37	0.57	20	0.98	78
2	Eicosanol	4.91	63	9.21	56	7.81	66	3.53	37	3.14	19	9.89	19	5.28	24	6.29	51
3	Heneicosanol	0.19	92	0.41	58	0.00	0	0.14	137	0.20	87	0.25	121	0.17	92	0.20	100
4	Docosanol	20.43	55	27.35	39	21.36	43	10.81	19	24.74	23	32.78	32	22.98	16	22.54	37
5	◇	0.09	224	0.49	76	0.00	0	0.25	61	0.31	87	0.14	200	0.41	29	0.25	92
6	Tricosanol	0.09	224	0.45	61	0.36	115	0.20	92	0.30	90	0.29	134	0.40	10	0.30	90
7	Tetracosanol	7.66	23	9.87	37	10.74	42	6.66	30	18.40	38	17.24	8	13.81	22	11.52	30
8	Squalene	0.00	0	0.00	0	0.00	0	0.59	137	0.32	112	0.00	0	0.00	0	0.13	273
9	Tetracosanoic acid	0.00	0	0.33	126	0.18	200	0.00	0	0.28	88	0.15	200	0.39	22	0.19	134
10	Pentacosanol	0.00	0	0.17	95	0.00	0	0.11	138	0.27	88	0.12	200	0.10	137	0.10	149
11	Hexacosanol	0.62	71	1.10	60	1.63	103	0.95	28	1.81	34	1.70	37	1.02	30	1.20	63
12	◇	1.08	38	0.79	67	0.00	0	1.31	13	0.72	13	0.00	0	0.07	224	0.59	49
13	Triterpenic alcohol	6.20	42	2.65	47	3.48	30	4.44	31	3.27	37	3.34	50	4.04	38	3.99	41
14	◇	0.00	0	0.00	0	0.00	0	1.06	85	0.00	0	0.00	0	0.00	0	0.17	215
15	Triterpene	1.52	44	0.63	95	0.51	200	1.78	8	0.95	5	0.24	200	0.70	26	0.94	58
16	◇	0.00	0	0.96	107	0.00	0	0.00	0	0.00	0	0.00	0	0.26	141	0.20	228
17	◇	0.94	75	1.25	70	0.60	200	0.76	96	0.33	88	0.71	157	0.86	75	0.82	103
18	Triterpene	2.95	58	0.21	223	0.00	0	3.73	16	1.70	12	0.16	200	1.23	63	1.49	56
19	Friedelin	15.98	34	15.41	37	14.66	17	23.38	15	11.40	60	14.23	37	19.33	31	16.78	31
20	Triterpene	14.33	17	9.22	71	8.41	69	9.11	18	12.41	70	4.57	58	11.44	25	9.99	46
21	Betulin	8.75	34	8.47	40	6.50	85	13.86	14	11.65	31	5.57	70	9.56	16	9.24	36
22	◇	0.31	149	0.14	224	0.00	0	1.12	115	0.11	173	0.00	0	0.00	0	0.26	218
23	Triterpene	1.82	75	0.11	224	0.00	0	1.66	14	0.59	24	0.00	0	0.72	67	0.75	81
24	Triterpene	0.71	93	0.42	161	0.00	0	0.72	62	0.21	90	0.00	0	0.18	153	0.35	128
25	Triterpene	2.00	56	0.48	141	0.00	0	2.15	30	1.26	55	0.00	0	1.12	19	1.05	60
26	Cerin	6.92	49	3.22	40	2.00	72	10.25	17	2.98	31	5.57	85	4.01	31	5.20	47

1. Almadén de la Plata; 2. Los Barrios; 3. Medina Sidonia; 4. El Chaparral; 5. Jerez de los Caballeros; 6. Maçanet de Cabranys; 7. Forallac. Average (x) and coefficient of variation (CV) were calculated for 5 samples in Almadén de la Plata, Los Barrios, El Chaparral and Forallac, 4 samples in Medina Sidonia and Maçanet de Cabranys and 3 samples in Jerez de los Caballeros. ϶ Average and CV were calculated for the whole 31 samples. ◇ Unidentified

Table 4. GC-MS analysis of acid fraction of waxes of *Quercus suber* reproduction cork from different Spanish provenances. Concentrations are expressed as area % of the total derivative peak

		1		2		3	
		x	CV	x	CV	x	CV
1	◇	0.00	0	2.08	143	0.31	200
2	Tetradecanoic acid	0.21	114	0.77	54	0.80	23
3	Pentadecanoic acid	0.12	156	0.12	155	0.00	0
4	12-Hexadecenoic acid	0.27	92	0.85	49	0.52	136
5	Hexadecanoic acid	6.64	23	8.63	45	5.28	28
6	9-Heptadecenoic acid	0.07	224	0.03	224	0.11	200
7	12-Heptadecenoic acid	0.00	0	0.02	224	0.34	118
8	Heptadecanoic acid	0.33	67	0.64	59	0.12	200
9	9,12-Octadecadienoic acid	3.92	60	4.08	54	4.02	61
10	9-octadecenoic acid	5.12	41	3.18	42	1.79	51
11	Octadecanoic acid	4.36	25	5.30	52	3.19	45
12	Eicosanol	0.00	0	0.43	106	0.66	75
13	11-Eicosenoic acid	0.34	86	0.03	224	0.34	130
14	18-Hydroxy-9,12-octadecadienoic acid	0.11	173	0.03	224	0.00	0
15	Eicosanoic acid	1.68	27	2.20	51	1.98	23
16	Heneicosanoic acid	0.08	139	0.12	172	0.08	200
17	18-Hydroxy-9-octadecenoic acid	2.06	65	1.42	45	1.01	22
18	Docosanoic acid	8.12	35	8.15	33	7.09	9
19	Tetracosanol	0.28	116	0.18	224	0.00	0
20	Hydroxyacid	1.29	48	0.94	50	1.34	29
21	◇	0.82	32	0.54	103	0.00	0
22	Tetracosanoic acid	2.48	36	1.29	25	1.62	29
23	Hydroxyacid	19.77	24	26.04	32	23.66	9
24	◇	1.61	21	0.54	60	0.91	22
25	Hydroxyacid	3.61	14	1.86	23	2.90	46
26	◇	0.75	90	2.64	172	0.00	0
27	Triterpene + Hydroxyacid	0.12	153	0.25	184	0.32	200
28	◇	0.74	91	0.14	194	0.93	154
29	Triterpene + Hydroxyacid	0.72	69	0.32	166	0.65	136
30	◇	0.47	155	0.32	164	0.88	128
31	Betulin	2.48	174	1.94	25	1.96	73
32	Triterpene	28.99	41	22.94	37	31.27	28
33	◇	1.45	40	0.13	224	0.83	163

1. Almadén de la Plata; 2. Los Barrios; 3. Medina Sidonia; 4. El Chaparral; 5. Jerez de los Caballeros; 6. Maçanet de Cabranys; 7. Forallac. Average (x) and coefficient of variation (CV) were calculated for 5 samples in Almadén de la Plata, Los Barrios, El Chaparral and Forallac, 4 samples in Medina Sidonia and Maçanet de Cabranys and 3 samples in Jerez de los Caballeros. э Average and CV were calculated for the whole 31 samples. ◇ Unidentified

cessing study (Conde et al., 1999). Both considering the global average value or the individual population contents of each component, the most abundant alcohol was docosanol, followed by tetracosanol and eicosanol. The global alcohols contents for each population were 34.28% in 1, 49.69% in 2, 44.39% in 3, 22.78% in 4, 49.45% in 5, 63.84% in 6 and 44.33% in 7. Within the triterpenes group (49.91%, global average value), 11 components were detected. Friedelin was the most abundant, followed by the triterpene 20 and betulin, with similar percent-

Table 4. Continuation (For legend see page 276)

4		5		6		7		Total э		
x	CV	x	CV	x	CV	x	CV	x	CV	
0.00	0	0.00	0	0.51	116	0.00	0	0.44	283	1
0.06	224	0.55	64	0.43	84	0.38	97	0.44	70	2
0.00	0	0.22	96	0.42	72	0.21	158	0.15	143	3
0.30	61	1.10	55	0.45	150	0.40	98	0.53	89	4
6.58	26	8.03	19	5.30	14	1.57	34	5.92	34	5
0.00	0	0.00	0	0.76	23	0.03	224	0.13	92	6
0.00	0	0.41	94	0.25	200	0.00	0	0.12	213	7
0.38	60	0.58	44	0.53	69	0.00	0	0.36	74	8
4.34	39	6.02	29	3.67	5	1.19	38	3.76	48	9
6.02	27	8.17	56	3.72	24	1.54	55	4.06	46	10
4.55	31	4.17	63	3.23	23	1.00	33	3.68	45	11
0.48	144	0.00	0	0.54	75	1.13	224	0.32	131	12
0.21	93	0.00	0	0.97	30	0.00	0	0.26	90	13
0.06	224	0.00	0	0.37	123	0.00	0	0.08	238	14
1.38	26	1.83	73	2.12	16	0.76	31	1.68	41	15
0.21	57	0.24	90	0.30	118	0.12	224	0.16	136	16
0.75	23	1.45	45	1.39	14	0.50	78	1.21	55	17
4.93	95	5.57	47	8.48	9	6.44	26	7.01	26	18
0.00	0	0.09	173	0.37	82	0.11	224	0.15	176	19
0.58	23	0.71	51	1.13	72	0.61	37	0.94	50	20
0.54	18	0.10	173	0.00	0	0.21	165	0.35	85	21
1.78	19	1.59	55	2.78	18	1.37	15	1.84	27	22
13.11	9	19.12	31	32.63	22	41.64	9	25.33	21	23
1.90	33	0.95	30	1.03	25	0.85	31	1.13	32	24
4.08	32	2.71	29	3.61	13	3.18	47	3.16	32	25
0.80	65	0.15	173	0.00	0	0.00	0	0.69	273	26
2.90	70	0.00	0	0.62	94	0.14	224	0.67	137	27
3.00	52	0.85	88	0.47	77	0.15	224	0.91	100	28
1.77	51	0.42	105	0.10	200	0.19	139	0.65	95	29
0.00	0	1.16	62	0.74	90	0.22	224	0.48	136	30
6.13	28	0.97	45	2.30	88	3.11	31	3.03	71	31
28.63	22	22.18	39	14.78	53	27.97	39	25.59	36	32
1.63	19	0.33	89	0.15	200	0.08	224	0.67	86	33

For explanations see page 276

ages, and, finally, cerin. This group of components reached the following percentages in the different provenances: 61.18% in 1, 40.82% in 2, 35.55% in 3, 71.66% in 4, 46.74% in 5, 33.67% in 6 and 52.34% in 7. The scarce presence of monocarboxylic fatty acids (0.19%, global average value) is represented by the tetracosanoic acid, accompanied only in some samples by traces of the hexadecanoic acid. The percentage of identified components in the neutral fraction amounts to 93.23% of the total percentage of the components' derivative peaks.

The results of the composition of the waxes acid fraction are arranged in Table 4 for the different provenances studied. The fatty acids group (global average value 61.47%), includes the saturated even C_{14}–C_{24} (although the C_{12} member, previously identified – Conde et al., 1999 – was not found) and odd C_{15},

C_{17}, C_{21} members, accompanied by a great amount of unsaturated terms and some ω-hydroxyacids, 18-hydroxy-9,12-octadecadienoic and 18-hydroxy-9-octadecenoic acids. The most abundant components of this group are the docosanoic and hexadecanoic acids and the three C_{18} acids without terminal hydroxyl group, 9-octadecenoic, 9,12-octadecadienoic and octadecanoic acids. The contents of fatty acids in each provenance were 61.09% in 1, 66.00% in 2, 56.67% in 3, 51.66% in 4, 62.69% in 5, 72.90% in 6 and 61.08% in 7. Four triterpenes were detected in the acid fraction (29.29%, global average value), although in these samples the most important triterpenic component was the triterpene 32. The percentages of this group of components in the seven populations considered were 31.99% in 1, 25.16% in 2, 33.71% in 3, 37.10% in 4, 25.35% in 5, 17.43% in 6 and 31.24% in 7. In the acid fraction of the waxes of these samples, only C_{20} and C_{24} alcohols were found (the C_{28} member, identified in some samples of the industrial processing study – Conde et al., 1999 – was not detected). The global average value for the alcohols in the acid fraction was 0.47%, and in the different provenances: 0.28% in 1, 0.61% in 2, 0.66% in 3, 0.48% in 4, 0.09% in 5, 0.91% in 6 and 0.24% in 7. The percentage of identified components in the acid fraction amounts to 91.23% of the total percentage of the components' derivative peaks.

The average global values were: 0.19% and 61.47% of fatty acids and 43.12% and 0.47% of alcohols for the neutral and acid fractions, and relating them to cork, 1.06% of acids and 2.51% of alcohols. These values are higher than those found by us in the samples of our study on the industrial processing influence and those reported in the literature: 0.7% of fatty alcohols in cork (Bescansa López et al., 1966); 45.8% of fatty acids in the waxes (Pes, 1974), and higher than 33% of fatty acids in the waxes, described by Graça and Pereira (1995). The percentage of triterpenes, calculated as exposed before in relation to cork, was 3.39%.

Tables 5 and 6 include the results of the significance levels of the pairwise *T*-test of the neutral and acid fractions components grouped by provenances.

When the three groups of components (fatty acids and alcohols and triterpenes) identified in the neutral fraction of the waxes were considered (Tables 3 and 5), significant differences were found among the provenances in the contents of alcohols and triterpenes, mainly in this last group, although the differences in the acid contents were neglible. The most pronounced significant differences were obtained in the comparisons among the populations 4 *versus* 6, 3 and 2, on one side, and 1 *versus* 6, on the other one. Since these concentrations are expressed as relative percentages of each component in the chromatogram, these differences are connected with the fact that the 4 and 1 populations showed the highest contents of triterpenes and, therefore, the lowest contents of alcohols, whereas 6, 3 and 2 showed the maximum contents of alcohols and the minimum contents of triterpenes. Moreover, it must be pointed out that the populations from Cádiz did not present significant differences in the contents of alcohols and triterpenes among themselves and when both were compared with that of Badajoz (2 *versus* 3, 2 *versus* 5 and 3 *versus* 5). When differences were studied concerning the individual components of this fraction, similar results were obtained. Among the components of the first part of the chromatogram (1–11), which were fatty alcohols and some acids, there was a lower number of differences than among those components in the second part of the chromatogram (13–26), consisting of triterpenes, with the exception of hexacosanol, that presented a very important contribution to the differences among populations. Generally, the comparisons among populations that showed a higher number of components with significant differences were those of 4 *versus* 2, 3, 5, 6 and 7. This means that the population 4

Table 5. Significance levels of pairwise *T*-test of all the neutral fraction components grouped by provenances (variances are not assumed to be equal)

		1 *versus* 6	2 *versus* 4	3 *versus*		4 *versus*			5 *versus* 7	6 *versus* 7
				4	7	5	6	7		
1	Octadecanol									
2	Eicosanol									
3	Heneicosanol									
4	Docosanol									
5	◇									
6	Tricosanol									
7	Tetracosanol	***					**			
8	Squalene						*			
9	Tetracosanoic acid									
10	Pentacosanol									
11	Hexacosanol									
12	◇			**				**	**	
13	Triterpenic alcohol						**	*		
14	◇					**				
15	Triterpene					*				
16	◇							**		
17	◇							*		
18	Triterpene		***	**						
19	Friedelin						**			
20	Triterpene						*			
21	Betulin									
22	◇									
23	Triterpene		***	**						
24	Triterpene					**				
25	Triterpene				**		**			**
26	Cerin		**	**						
						**				
								**		

1. Almadén de la Plata; 2. Los Barrios; 3. Medina Sidonia; 4. El Chaparral; 5. Jerez de los Caballeros; 6. Maçanet de Cabranys; 7. Forallac. *** 0.1% significance; ** 1% significance. ◇ Unidentified

presents clear differences with the remaining ones, except with 1, both in the global contents and in the percentages of the individual components.

Concerning the differences among the provenances in the contents of the groups of fatty acids as well as alcohols and triterpenes in the acid fraction (Tables 4 and 6), a higher number of differences were obtained in the comparisons of the contents of acids and triterpenes, whereas the differences were practically null in the case of the alcohols. It can be pointed out that population 6 showed the highest contents of acids and the lowest of triterpenes, and presented significant differences with population 4, characterised by the lowest contents of acids and the highest of triterpenes. Population 7 also showed significant differences compared to populations 4 and 5. The relationship between the individual component concentrations and the total content of each group of components was not so clear as in the neutral fraction. In this case, the differences

Table 6. Significance levels of pairwise *T*-test of all the acid fraction components grouped by provenances (variances are not assumed to be equal)

		1 *versus*		2 *versus* 4	3 *versus*		4 *versus*		5 *versus* 7
		2	7		4	7	6	7	
1	◇								
2	Tetradecanoic acid								
3	Pentadecanoic acid								
4	12-Hexadecenoic acid								
5	Hexadecanoic acid								**
6	9-Heptadecenoic acid								
7	12-Heptadecenoic acid								
8	Heptadecanoic acid								
9	9,12-Octadecadienoic acid								**
10	9-octadecenoic acid								
11	Octadecanoic acid								
12	Eicosanol								
13	11-Eicosenoic acid								
14	18-Hydroxy-9,12-octadecadienoic acid								
15	Eicosanoic acid								
16	Heneicosanoic acid								
17	18-Hydroxy-9-octadecenoic acid								
18	Docosanoic acid								
19	Tetracosanal								
20	Hydroxyacid								
21	◇				**		**		
22	Tetracosanoic acid								
23	Hydroxyacid		**			**		**	
24	◇							*	
25	Hydroxyacid	**							
26	◇								
27	Triterpene + Hydroxyacid								
28	◇								
29	Triterpene + Hydroxyacid								
30	◇								
31	Betulin								
32	Triterpene								
33	◇			**			**		
								**	

1. Almadén de la Plata; 2. Los Barrios; 3. Medina Sidonia; 4. El Chaparral; 5. Jerez de los Caballeros; 6. Maçanet de Cabranys; 7. Forallac.*** 0.1% significance; ** 1% significance. ◇ Unidentified

were scarcer and did not show a unique pattern, neither in the components, nor in the populations.

In both cases, no clear relationship was found between the geographical proximity of the provenances and chemical similarity. Although there are logical resemblances between populations of provenances nearest to each other, such as 2 and 3, both from Cádiz, there are other provenances, such as 4 and 5, both from Extremadura, or 6 and 7, from Gerona, very close but chemically different.

The minor components of both fractions (mainly those whose percentages were less than 0.5%) showed a very variable behaviour, indicated by their global coefficients of variation and by those corresponding to the groups of samples of each population.

In the stepwise discriminant analysis among populations, considering the percentages of each component from the neutral fraction as variables, five components providing the largest discrimination were selected, step by step: tetracosanol, triterpenes 18 and 20 and the unidentified components 5 and 12. The resulting mathematical model explained 100% of the total dispersion that was distributed among five canonical variables. Figure 1 is a graphical representation of the projections of the points of each group on the plane defined by the two principal canonical axes that represent a cumulative proportion of 75% of the total dispersion. The sets of points of the 4 and 1 populations are clearly separated from the ensemble of 2, 3, 5, 6 and 7, although those of 2 were slightly displaced from this global ensemble. This pattern of distribution coincides with the results obtained in the univariate analysis of the neutral fraction components.

When the discriminant analysis was carried out considering the components of the acid fractions, four components were selected as those providing the greatest discrimination among populations: 9-heptadecenoic acid, the hydroxy-acid (component 23), and the unidentified components 24 and 33. The resulting mathematical model also explained the 100% of the total dispersion, that was distributed among four canonical variables. The graphical representation originated at the end of the statistical analysis is Fig. 2 representing a cumulative proportion of 95% of the total dispersion. In this Figure, the set of points of the 6 population was completely separated from the group including all the remaining provenances.

From the above results, it can be concluded that, although the contents of waxes and of neutral and acid fractions did not show significant differences

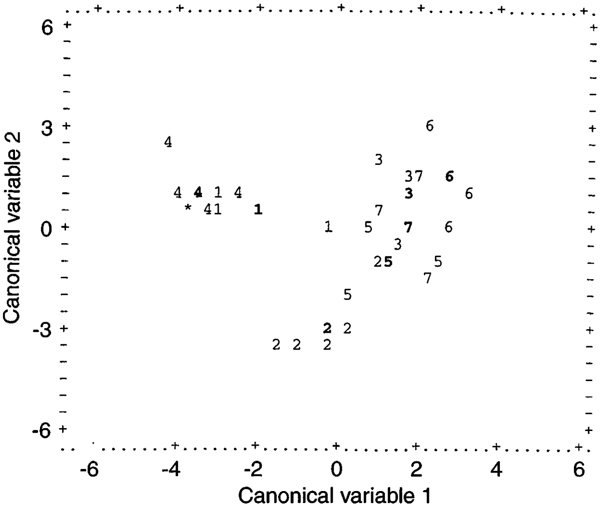

Fig. 1. Stepwise discriminant analysis of the neutral fraction components. Projections of the points of each provenance on the two principal canonical axes. 1 = Almadén de la Plata; 2 = Los Barrios; 3 = Medina Sidonia; 4 = El Chaparral; 5 = Jerez de los Caballeros; 6 = Maçanet de Cabranys; 7 = Forallac. **1**, **2**, **3**, **4**, **5**, **6** and **7** are the group centroids for each population, respectively

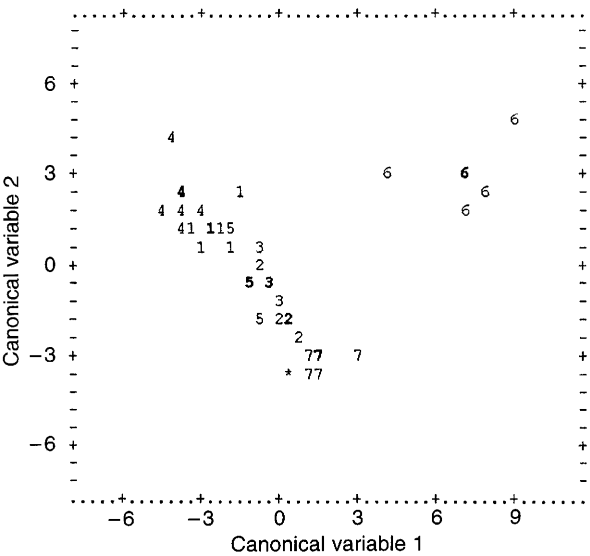

Fig. 2. Stepwise discriminant analysis of the acid fraction components. Projections of the points of each provenance on the two principal canonical axes. 1 = Almadén de la Plata; 2 = Los Barrios; 3 = Medina Sidonia; 4 = El Chaparral; 5 = Jerez de los Caballeros; 6 = Maçanet de Cabranys; 7 = Forallac. **1, 2, 3, 4, 5, 6** and **7** are the group centroids for each population, respectively

among the populations of the different studied provenances, their individual components (fatty alcohols and acid and triterpenes) presented an important inter- and intra-populational variability. There is no agreement in the differences among provenances considering the components of the neutral fraction or those of the acid fraction. No clear relationship was found between geographical proximity of the provenances and chemical similarity of the waxes composition.

References

Bescansa-López JL, Ribas-Marqués I (1966) Química del corcho. XVI. Hidrocarburos parafínicos en el corcho. An. Soc. Esp. Fís.-Quím. 62-B: 871–873

Bescansa-López JL, Gil Curbera G, Ribas-Marqués I (1966) Química del corcho. XV. Alcoholes parafínicos del corcho. 1. An. Soc. Esp. Fís.-Quím. 62-B: 865–869

Caldas MM, Ferreira JML, Borges MA (1985) Abordagem sobre a caracterizaçao química da cortiça nas várias etapas do processamento industrial. Cortiça 560: 549–560

Carvalho JS (1968) Química da cortiça. Boletim JNC. 357: 3–11

Cadahía E, Conde E, Fernández de Simón B, García-Vallejo MC (1996) Proanthocyanidins and ellagitannins in cork of *Quercus suber.* In: Vercauteren J, Cheze C, Dumon MC, Weber JF (Eds.). Polyphenols Communications 96, pp. 215–216, Bordeaux

Conde E, Cadahía E, García-Vallejo MC, Fernández de Simón B, González-Adrados JR (1997) Low molecular weight polyphenols in cork of *Quercus suber.* J. Agric. Food Chem. 45: 2695–2700

Conde E, García-Vallejo MC, Cadahía E (1999) Waxes composition of reproduction cork from *Quercus suber* and its variability throughout the industrial processing. Wood Sci. Technol. (in press)

García-Vallejo MC, Conde E, Cadahía E, Fernández de Simón B (1997) Suberin composition of reproduction cork from *Quercus suber.* Holzforschung, 51: 219–224

Graça J, Pereira H (1995) The acidic fraction of cork lipophilic solubles. In: Proccedings of the Procork Workshop 2 "European Research on Cork", p. 2. Lisboa

Guillemonat A (1960) Progrès récents dans l'étude de la constitution chimique du liège. Ann. Fac. Sci. 30: 43–54

Hata K, Sogo M, Fukuhara T, Hochi M (1969) On the suberin of the outer bark of some Japanese tree species. Techn. Bull. Agric. Kagawa Univ. 20: 112–119

Holloway PJ (1972) The composition of suberin from the corks of *Quercus suber* L. and *Betula pendula* Roth. Chem. Phys. Lipids 9: 158–170

Jennhrich R, Sampson P (1985) P7M. Stepwise Discriminant analysis. In: Dixon WJ (Ed.). BMDP Statistical Software, p. 519. Los Angeles: University California Press, Berkeley

Marcos de Lanuza J (1968) Estudios sobre el corcho de *Quercus suber*. Instituto Forestal de Investigaciones y Experiencias. Madrid

Pereira H (1979) Constituiçao química da cortiça. Estado actual dos conhecimientos. Cortiça 483: 259–264

Pereira H (1988) Chemical composition and variability of cork from *Quercus suber* L. Wood Sci. Technol. 22: 211–218

Pes A (1974) Primo contributo alla conoscenza delle caractteristiche chimico-fisiche del sughero verdonato. Memoria Satz. Sper. Sughero 41

Pes A, Lissia F (1972) Estrazione di cere e di acidi grassi dal sughero. I. Un impianto piloto in vetro Duran 50. Collana Technologica 5, Stazione Sper. Sughero