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Effects of time of grape pomace fermentation and distillation cuts on the chemical composition of grape marcs

Abstract The effects of fermentation time and distillation cuts on the composition of distillates in terms of ethanal, 1,1-diethoxyethane, methanol, 1-butanol, 2-butanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl acetate, ethyl 2-hydroxypropanoate, 3-methylbutyl acetate, hexyl acetate, 2-phenylethyl acetate, hexanol, *trans*-2-hexenol, *trans*-3-hexenol, *cis*-3-hexenol, 2-phenylethanol, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, 2-methylpropanoic acid, 3-methylbutanoic acid, hexanoic acid, octanoic acid, decanoic acid, and dodecanoic acid were assessed through data generated according to a factorial design using analysis of variance and principal component analysis. Four times of storage of pomace obtained following winemaking of two grape varieties of white Verde wine (Alvarinho and Loureiro) and three distillation cuts were considered; volatile compounds in the 24 samples generated were analyzed directly, and indirectly after extraction and concentration, by capillary gas chromatography. The results generated have suggested clear differences ($P<0.05$) between distillate cuts obtained throughout fermentation times for each grape variety. The major differences between the different distillate fractions analyzed were accounted for by the contents of diethyl butanoate, ethyl 2-hydroxypropanoate, and the sum of 3-methylbutanoic and 2-methylpropanoic acids for Loureiro, whereas the main differences were accounted for by the contents of diethyl butanoate and the sum of the carboxylic acids for Alvarinho.

Key words Bagaceira · Grape pomace · Distillation · Volatiles

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Introduction

Bagaceiras are a unique class of spirits, produced from the distillation of grape pomace, which are rather flavorful. After having pressed the grapes in the winemaking process, the grape pomace is, in Mediterranean countries, often stored under sealed conditions for a given period of time in order to promote spontaneous anaerobic fermentation of residual sugars. The various volatile organic components thus formed are then recovered by steam distillation, or by distillation after having added water, in stills made of copper [1]. Silva and Malcata [2] have empirically assessed the effects of adding tartaric acid, adding pectinases, using containers built of different materials, and allowing fermentation to occur for different periods; the time of fermentation accounted for the most important statistical effect, and such realization provided an impetus for this paper as an attempt to further characterize the effect of this variable on the chemical composition of bagaceiras, especially with respect to compounds whose levels have legal implications in terms of public trade.

When batch distillation is selected, as is often the case, two sequential cuts of distillate are consistently and deliberately made, viz. a first cut to separate the head products from the heart products, and a second cut to separate the heart products from the tail products; since only the heart products have commercial value, the head and tail products are eventually subject to a separate distillation for non-food purposes. The head fraction, or the first fraction of condensate (also termed cabeças), is very rich in methanol; the heart fraction, or the second fraction of condensate (also termed coração), is a complex mixture of volatiles, most of which contribute organoleptic keynotes; the tail fraction, or the last fraction of condensate (also termed caudas), is rich in less volatile compounds [3–6]. The definition of the times when the distillation cuts are to be made is, however, still done in an empirical fashion, based only on the winemaker's experience and on the bulk concentration of alcohols as assessed by densitometry; the cut between head and heart products is typically made between 70% and 80% (v/v) ethanol whereas the cut between heart and tail products is typically made between 35% and 50% (v/v) ethanol.

Awareness of the major organoleptic differences between the three distillation fractions has also provided a motivation for this study in attempts to determine whether statistically significant differences exist between the chemical composition of the different fractions of distillates obtained after fermentation of grape pomace from two different grape varieties of Verde white wine.

Materials and methods

Pomace substrate

Grapes of the Alvarinho variety were obtained from Adega Cooperativa de Monção (Monção, Portugal), whereas grapes of the Loureiro variety were obtained from Direcção Regional de Agricultura de Entre-Douro e Minho (Vairão, Portugal); both grape varieties were independently used to manufacture Verde white wine. Appropriate plastic containers were then filled with 150 kg of grape pomace left after winemaking, duly sealed to ensure anaerobic aconditions, and maintained at room temperature (ca. 20 °C) for periods of up to 12 weeks.

Distillation

After anaerobic storage for 3, 6, 9, and 12 weeks, the full load of each aforementioned container was steam distilled in copper batch stills to produce ca. 7.5 l of spirits. Distillation products were collected as three fractions: cabeças (overall volume, V, equal to 2 l), coração (V=3.5 l), and caudas (V=2 l); these fractions correspond to levels of total alcohols of ca. 90% (v/v), 60% (v/v), and 30% (v/v) respectively. Distillates were collected in amber bottles, stoppered, and stored at 4 °C before analysis.

Chemical analyses

Chemicals. Standards (chromatographic grade) of alcohols (methanol, 2-butanol, 1-propanol, 2-methyl-1-propanol, 1-propenol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, hexanol, *trans*-3-hexenol, *cis*-3-hexenol, *trans*-2-hexenol, 2-phenylethanol, 3-octanol and 4-methyl-2-pentanol) and esters (ethyl acetate, ethyl butyrate, 2-methylbutyl acetate, ethyl hexanoate, hexyl acetate, ethyl 2-hydroxypropanoate, ethyl octanoate, ethyl decanoate, diethyl butanoate, 2-phenylethyl acetate, and ethyl dodecanoate) were purchased from Merck (Schuchardt, Switzerland). Standards of carboxylic acids (2-methylpropanoic, 3-methylbutanoic, hexanoic, heptanoic, octanoic, decanoic, and dodecanoic acids), 1,1-diethoxyethane, and ethanal were purchased from Sigma (St. Louis, Mo., USA). Diethyl ether, hexane, and sulfuric acid were obtained from Merck.

Chromatographic assays. For assays of alcohols, 1,1-diethoxyethane, ethyl acetate, and ethanal (which are present at relatively high concentrations), a sample of 5 ml of every distillate fraction was mixed with 50 μl of an internal standard solution of 50 mg/l of 4-methyl-2-pentanol in ethanol [7]. The injector of the chromatograph (Perkin Elmer, Norwalk, Pa., USA) was maintained at 200 °C and operated under split mode. Elution was achieved in a 50 m×0.25 mm×0.2 μm capillary column (CPWAX 57CB; Chrompack, The Netherlands). The oven temperature program was as follows: 40 °C for 5 min, a linear ramp from 40 °C to 200 °C at 3 °C/min, and 200 °C for 20 min. Detection was by flame ionization (FID) at a temperature of 200 °C. Helium was used as the carrier gas at a split ratio of 1:60.

For assays of esters and fatty acids (which are present at relatively low concentrations), a sample of 10 ml of every distillate fraction was mixed with 40 ml of deionized water in order to lower the total alcohol content below 10% (v/v), and the resultant solution was mixed with 2 ml of an internal standard solution of 40 mg/l of 3-octanol in ethanol and 2 ml of an internal standard solution of 90 mg/l of heptanoic acid in ethanol. The pH was adjusted to 2.0 using a few drops of concentrated sulfuric acid. The mixture was extracted for 5 min with 4 ml of a 50% (v/v) mixture of diethyl ether and hexane, and this procedure was repeated twice with 2 ml of the same solvent [8]. The injector of the chromatograph was maintained at 250 °C and operated under splitless mode. Elution was achieved in a 25 m×0.32 mm×0.3 μm capillary column (FFAP CB-Wcot fused silica; Chrompack). The oven temperature program was as follows: 40°C for 5 min, a linear ramp from 40 to 220 °C at 3 °C/min, and 220 °C for 20 min. Detection was by flame ionization at a temperature of 250 °C. Helium was used as the carrier gas at a split ratio of 1:30.

In both situations, aliquots of 0.5 μl were automatically injected into the chromatograph. The carrier gas flew at 2 ml/min, and the gases required by the FID were supplied at pressures of 90 kPa (H_2) and 110 kPa (air). Integration was done using Omega software (PE Nelson, Norwalk, Pa., USA), taking as reference the peak areas corresponding to the aforementioned internal standards. Analyses were carried out in triplicate, and their average was used as a single data point in the Results and discussion section.

Statistical analyses

Analysis of variance. The effects of the technological parameters were assessed via analysis of variance (ANOVA), using time of fermentation and fraction of distillate (cabeças, coração, and caudas) as independent variables, with the aid of Statview software (Abacus Concepts, Berkeley, Calif., USA). All possible pairwise comparisons were done using a multiple t-statistic (Fisher's protected least significance difference test). Previous diagnostics of residuals have indicated that a constant variance and an independent and normal distribution of experimental errors existed, and so statistical validity of the aforementioned ANOVA methodology was ensured.

Principal components analysis. Attempts to reduce the number of independent dimensions in the sample space generated in our experimental program were done by principal components analysis (PCA), aiming at simplification of the approach without loss of relevant information, and at improvement of the associated understanding via identification of new, uncorrelated variables [6]. The STAT-ITCF software (Bordeaux, France), used for calculation of principal components, was applied to the multivariate data pertaining to all samples corresponding to different fractions of distillate, times of fermentation, and grape varieties, and eventually led to definition of linear combinations of variables that could account for most of the variation among the samples.

Results and discussion

Compositional data

Typical chromatograms of the samples analyzed are included in Figs. 1 and 2. The results obtained from quantification of the peaks in the chromatograms pertaining to the evolution with storage time of the concentrations of major volatile components in the three distillate frac-

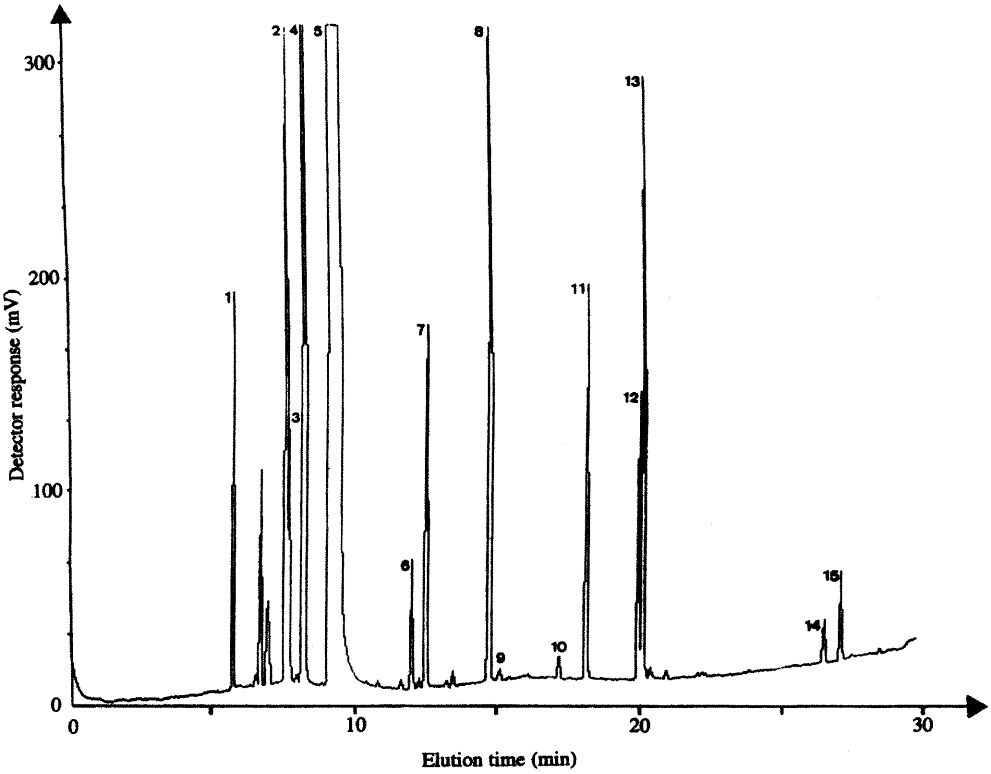

Fig. 1 Typical gas chromatogram obtained after direct injection of coração of a bagaceira from Loureiro. *1* ethanal; *2* ethyl acetate; *3* 1,1-diethoxyethane; *4* methanol; *5* ethanol; *6* 2-butanol; *7* 1-propanol; *8* 2-methyl-l-propanol; *9* 1-propenol; *10* 1-butanol; *11* 4-methyl-2-pentanol (internal standard); *12* 2-methyl-1-butanol; *13* 3-methyl-1-butanol; *14* ethyl 2-hydroxypropanoate; *15* hexanol

tions are depicted in Tables 1 and 2 for Alvarinho and Loureiro grape varieties, respectively. The experimental data were grouped according to the main chemical classes of compounds identified (alcohols, esters, acids, and carbonyl compounds) and their relative abundance within each main class; alcohols were subgrouped as fermentative and non-fermentative (methanol, hexanol, and hexenols); acids were subgrouped in free fatty acids and volatile fatty acids (2-methylpropanoic and 3-methylbutanoic acids); and esters were subgrouped as those arising from the so-called organic acids (diethyl butanoate and ethyl 2-hydroxypropanoate) and acetates.

The levels of higher alcohols are influenced by several processing factors, namely grape variety, winemaking protocol, fermentation time and temperature, and distillation technique. The group of aliphatic alcohols (1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol) and aromatic alcohols (especially 2-phenylethanol) are quantitatively the largest group of flavor compounds. In the samples analyzed, their ranges are 358.07–1896.9 and 629.54–3048.5 mg/l for Alvarinho and Loureiro, respectively. Higher alcohols are contributed by the slightly fermented grapes themselves or are formed as a result of microbial action during anaerobic fermentation of the grape pomace; owing to their high volatilities, these compounds are concentrated in the first fraction of distillate (head fraction), where they are responsible for the strong and pungent smell and taste.

Free fatty acids are mainly produced via yeast metabolism of carbohydrates. Second to acetic acid, the most abundant acids in bagaceiras are hexanoic acid (up to 24 mg/l), decanoic acid (up to 23 mg/l), octanoic acid (up to 8 mg/l), and dodecanoic acid (up to 3 mg/l). Ranges for the sum of the concentrations of all free fatty acids were 5.7154–25.500 and 3.5410–18.600 mg/l for Alvarinho and Loureiro, respectively.

Fatty acid esters are qualitatively the largest group of aroma components. Although a particular key aroma can rarely be associated with a specific ester, they account collectively for an overall pleasant smell. Ethyl acetate, methyl acetate, and ethyl 2-hydroxypropanoate are the most abundant esters present in bagaceiras, with ranges of 186.84–2085.8, 0.00000–95.680, and 167.51–369.12 mg/l, respectively, for Alvarinho, and of 135.26–7684.4, 14.258–714.44, and 149.21–663.49 mg/l, respectively, for Loureiro. The ranges for the sum of the fatty acid ethyl ester contents for Alvarinho and Loureiro found were 3.2813–29.243 and 7.0095–44.112 mg/l, respectively.

Aldehydes are found in bagaceiras as a result of spontaneous, or microbially mediated, oxidation. More than 90% of the total aldehyde content is accounted for by ethanal and its diethyl acetal, 1,1-diethoxyethane; the contents of ethanal were in the ranges of 11.698–277.55 and 147.01–604.42 mg/l, whereas those of 1,1-diethoxyethane were in the ranges of 0.00000–142.81 and 25.653–895.89 mg/l, for Alvarinho and Loureiro varieties, respectively.

For Loureiro, the ethyl acetate, methyl acetate, ethyl hexanoate, octanoate, decanoate, and dodecanoate are distilled first, and are accordingly considered as head products. On the other hand, ethyl 2-hydroxypropa-

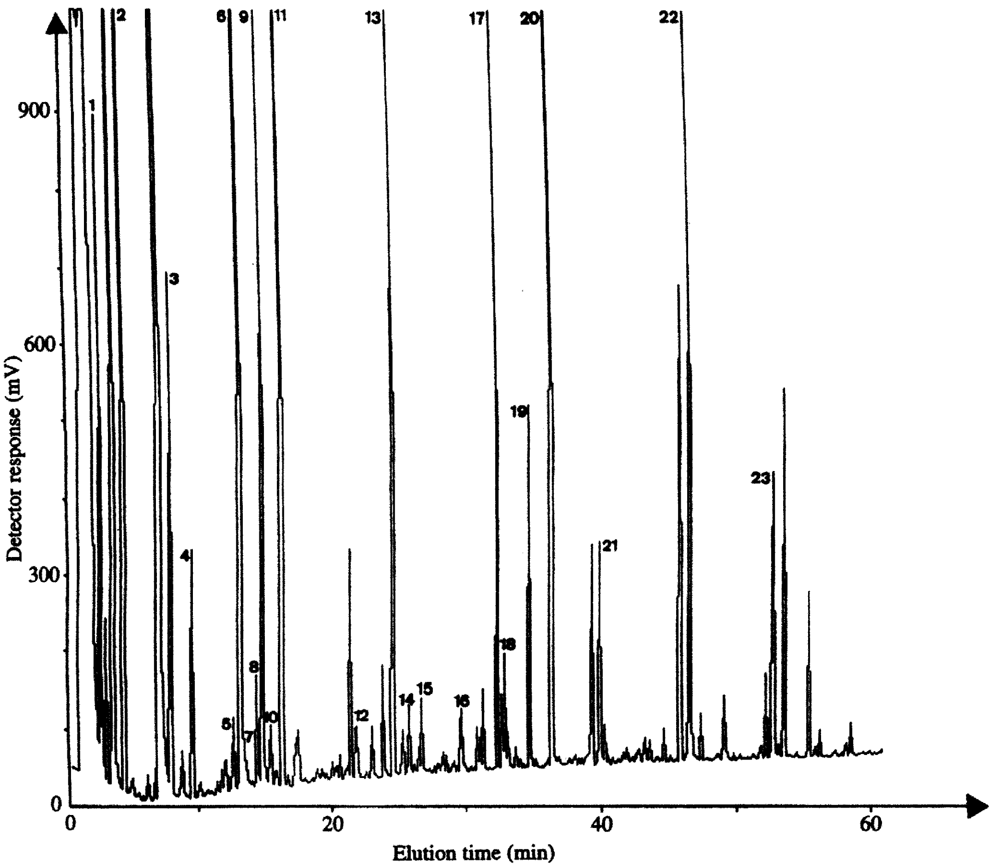

Fig. 2 Typical gas chromatogram obtained after injection of the diethyl ether/hexane extract of coração of a bagaceira from Loureiro. *1* ethyl butyrate; *2* 2-methylbutyl acetate; *3* ethyl hexanoate; *4* hexyl acetate; *5* ethyl 2-hydroxypropanoate; *6* hexanol; *7 trans*-3-hexenol; *8 cis*-3-hexenol; *9* 3-octanol (internal standard); *10 trans*-2-hexenol; *11* ethyl octanoate; *12* 2-methylpropanoic acid; *13* ethyl decanoate; *14* 3-methylbutanoic acid; *15* diethyl butanoate; *16* 2-phenylethylacetate; *17* ethyl dodecanoate; *18* hexanoic acid; *19* 2-phenylethanol; *20* heptanoic acid (internal standard); *21* octanoic acid; *22* decanoic acid; *23* dodecanoic acid

noate is found in both the heart and tail fractions. The results obtained for Alvarinho are not, however, in agreement with the above results, especially concerning ethyl 2-hydroxypropanoate; this component is at times present only as a tail product, or alternatively as a heart product, or even as a head product. Although, in principle, it would be expected that a given component should be classified either as a head, a heart, or a tail product irrespective of grape variety, different patterns of heating during distillation, heterogeneity of the grape pomace, and the variable proportions of water added to the grape pomace immediately before distillation might account partially for a hybrid behavior.

The contents in terms of such specific volatiles as methanol and 2-butanol should be carefully and independently considered since their concentrations may provide an objective criterion for a definition of cuts between head, heart, and tail fractions. This is especially important because of toxicity (depending on the overall amount ingested and actual ratio to ethanol content, methanol may lead to blindness) and strong perception (the presence of 2-butanol is deleterious to quality because of its associated off-flavor). Methanol is formed by enzymes that split the methoxyl group from the pectin present in crushed grapes; the rate of degradation of pectins increases with the extent of maceration of the pomace and the total concentration of pectinases, whereas the concentration of methanol in the final bagaceira increases as the splitting between cabeças and coração is made earlier. The methanol content ranges were 1989.4–5486.5 and 9901.3–15407 mg/l for Alvarinho and Loureiro, respectively. There is a general consensus that 2-butanol originates from bacterial action, probably via metabolic routes starting upon 2,3-butanediol. In the cases where its value was above the analytical detection threshold, the contents were in the range of 0.00000–64.493 and 19.593–350.89 mg/l for Alvarinho and Loureiro, respectively.

In conclusion, it can be stated that the main differences between the various fractions of distillates are accounted for by the content of diethyl butanoate, ethyl 2-hydroxypropanoate, and the sum of the isoacids in the case of Loureiro, and diethyl butanoate and the sum of the carboxylic acids in the case of Alvarinho.

ANOVA data

The results of the ANOVA are depicted in Tables 3 and 4, whereas the results of Fisher's protected least significant difference tests after adequate pairing of the data are depicted in Tables 5 and 6 for Alvarinho and Loureiro varieties, respectively.

Inspection of Tables 3 and 4 indicates that both the manipulated variables time of fermentation (t) and fraction of distillate (f) had, in general, statistically significant effects on the chemical composition of distillates from Alvarinho and Loureiro at the 5% level of

Table 1 Mean concentrations of aldehydes, carboxylic acids, esters, and alcohols (mg/l) in distillates obtained from Alvarinho; head products: Cab; heart products: Cor; and tail products: Cau[a]

No.	Component	Fermentation time (weeks)											
		3			6			9			12		
		Cab	Cor	Cau	Cab	Cor	Cau	Cab	Cor	Cau	Cab	Cor	Cau
1	Methanol	4554.2	2637.5	2981.6	3346.3	2076.5	1989.4	3958.5	2740.3	2283.1	5486.5	3182.6	2573.1
2	Ethanal	277.55	80.540	11.698	86.748	92.680	67.588	143.82	115.73	74.385	176.43	114.48	101.44
3	Acetal	142.81	5.9325	13.768	13.813	0.00000	0.00000	45.475	0.00000	0.00000	79.215	4.3850	0.00000
4	2-Butanol	0.00000	5.0500	5.2275	14.607	7.9200	0.00000	30.750	17.638	15.743	64.493	36.133	30.925
5	1-Propanol	351.97	163.34	263.60	235.76	122.73	94.248	278.80	193.24	156.15	314.14	176.39	146.98
6	2-Methylpropanol	539.87	212.93	393.18	331.23	168.17	109.96	407.05	273.44	218.35	470.43	252.75	220.40
7	1-Butanol	10.478	5.3375	6.2275	6.6750	17.085	0.00000	5.7825	0.00000	18.663	9.2075	5.8925	0.00000
8	2-Methyl-1-butanol	236.70	86.873	168.55	154.96	66.683	48.273	164.12	112.16	86.853	208.20	111.90	88.235
9	3-Methyl-1-butanol	757.86	317.44	604.93	517.36	244.76	105.59	556.68	386.61	307.51	695.62	381.13	321.83
	Total alcohols (4–9)	1896.9	790.96	1441.7	1260.6	627.35	358.07	1443.2	983.08	803.27	1762.1	964.19	808.37
10	1-Propenol	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
11	Hexanol	113.61	51.935	94.675	94.475	38.135	25.858	75.798	50.270	41.943	84.965	47.253	41.645
12	*trans*-3-Hexenol	0.00000	0.00000	0.00000	0.28070	0.05480	0.00000	0.18330	0.05410	0.00000	0.11510	0.02410	0.04480
13	*cis*-3-Hexenol	1.9717	2.2647	0.26050	2.1332	0.96480	0.57700	0.61580	0.28020	0.06800	0.29110	0.39390	0.39180
14	*trans*-2-Hexenol	0.14770	0.09000	0.05830	2.1115	0.00000	0.14830	0.01110	0.15900	0.00000	0.03120	0.03280	0.33550
	total hexenols (12–14)	2.1194	2.3547	0.31880	4.5254	1.0196	0.72530	0.81020	0.49330	0.06800	0.43740	0.45080	0.77210
15	2-Phenylethanol	9.8127	13.341	10.884	9.4199	19.749	6.8475	12.340	12.784	12.230	15.469	6.9300	10.721
16	2-Methylpropanoic acid	0.48410	0.00000	0.44210	1.5374	1.6403	0.84590	2.6545	1.1558	0.85120	1.8886	1.8598	0.76690
17	3-Methylbutanoic acid	0.44810	4.6700	4.0878	0.69940	4.7926	0.6150	1.7878	3.4920	5.8072	1.1504	2.0180	4.3438
	Total isoacids (16–17)	0.93220	4.6700	4.5299	2.2368	6.4329	1.4609	4.4423	4.6478	6.6584	3.0390	3.8778	5.1107
18	Hexanoic acid	2.2501	3.0470	3.0124	1.8723	18.925	3.1744	3.0725	5.4476	3.7630	2.4974	10.522	6.9901
19	Octanoic acid	1.6655	2.5498	2.5204	2.6776	3.9740	1.7045	1.8572	2.2182	1.5084	2.8892	2.7719	1.9509
20	Decanoic acid	0.34800	4.5434	3.4572	3.7088	2.2554	1.3662	2.5282	1.4251	2.6139	1.2812	3.0518	1.9429
21	Dodecanoic acid	1.4518	0.56540	0.44730	1.1016	0.34570	0.33550	0.69030	0.43770	0.66190	1.4284	0.11350	0.20660
	Total carboxylic acids (18–21)	5.7154	10.706	9.4373	9.3603	25.500	6.5806	8.1482	9.5286	8.5472	8.0962	16.459	11.091
22	Ethyl acetate	1325.6	321.24	402.82	437.91	305.99	186.84	1310.2	685.58	408.27	2085.8	678.70	733.08
23	Methyl acetate	40.105	4.1725	0.00000	19.685	0.00000	0.00000	59.883	32.700	34.348	95.680	40.070	36.805
24	3-Methylbutyl acetate	9.8119	3.9587	1.4483	5.9533	2.3797	3.2766	1.8623	2.6802	1.5370	5.8473	7.9395	4.4985
25	Hexyl acetate	3.8929	1.4413	0.24770	2.6857	0.50340	1.6379	0.57680	0.91240	0.61470	2.0855	1.6248	1.5044
26	2-Phenylethyl acetate	0.10750	0.00000	0.03810	0.10180	0.00700	0.01000	0.27440	0.33020	0.05960	0.16740	0.01750	0.10270
	Total acetates (24–26)	13.812	5.4000	1.7341	8.7408	2.8901	4.9245	2.7135	3.9228	2.2113	8.1002	9.5818	6.1056
27	Ethyl butyrate	0.15240	0.20200	0.01890	0.08290	0.14550	0.05470	0.06950	0.08640	0.05240	0.28250	0.07050	0.10230
28	Ethyl hexanoate	9.8413	4.3586	1.7099	4.2206	1.9713	2.9065	2.0026	2.1086	1.2612	5.1708	8.3337	4.2057
29	Ethyl octanoate	13.252	7.3532	4.5189	6.0999	3.3940	3.7844	2.2273	2.7354	1.4847	3.9989	11.028	3.2366
30	Ethyl decanoate	3.9459	4.1779	2.2718	3.9353	0.63620	2.1431	1.2361	0.48170	0.4214	1.8374	3.5019	1.1916
31	Ethyl dodecanoate	2.0514	1.2010	0.86780	1.0529	1.6034	0.82280	0.73390	0.35850	0.06160	0.60960	0.56110	0.23140
	Total esters (27–31)	29.243	17.293	9.3873	15.392	7.7504	9.7115	6.2694	5.7706	3.2813	11.899	23.495	8.9676
32	Diethyl butanoate	2.2177	2.8909	2.4818	7.1478	11.913	7.2122	10.392	6.6380	6.0182	12.462	37.744	10.746
33	Ethyl 2-hydroxypropanoate	228.54	230.70	196.99	261.74	178.96	167.51	258.86	237.56	194.36	369.19	277.12	244.18

[a] Grouping of compounds within each family follows accepted methods based on role upon aroma

Table 2 Mean concentrations of aldehydes, carboxylic acids, esters, and alcohols (mg/l) in distillates obtained from Loureiro; head products: Cab; heart products: Cor; and tail products: Cau[a]

No.	Component	Fermentation time (weeks)											
		3			6			9			12		
		Cab	Cor	Cau	Cab	Cor	Cau	Cab	Cor	Cau	Cab	Cor	Cau
1	Methanol	14125	12839	11484	11871	11974	10628	14600	13330	9901.3	15407	14750	11414
2	Ethanal	604.42	265.29	405.78	385.38	258.76	306.56	473.08	269.48	147.01	418.45	277.72	249.68
3	Acetal	895.89	62.288	72.383	402.72	154.50	79.595	507.27	229.68	25.653	444.42	219.30	60.440
4	2-Butanol	350.89	138.12	47.500	364.85	252.08	106.60	309.98	62.700	19.593	575.36	370.28	194.58
5	1-Propanol	572.03	388.37	183.48	552.30	510.99	264.28	544.42	391.79	199.30	587.13	463.41	289.32
6	2-Methylpropanol	913.09	433.82	152.27	860.04	546.16	97.038	811.19	469.67	179.26	790.83	532.60	295.33
7	1-Butanol	23.075	16.725	10.273	22.290	20.318	15.268	25.340	23.308	11.758	24.068	20.360	12.640
8	2-Methyl-1-butanol	345.99	193.21	66.060	335.10	236.53	119.54	358.32	233.78	91.1223	323.28	228.37	124.90
9	3-Methyl-1-butanol	747.68	494.41	169.96	735.78	668.21	296.01	811.74	579.11	234.75	747.84	573.87	316.93
	Total alcohols (4–9)	2952.8	1664.7	629.54	2870.4	2234.3	898.73	2861.0	1760.3	735.78	3048.5	2188.5	1233.7
10	1-Propenol	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
11	Hexanol	105.37	94.780	34.333	106.52	100.93	50.613	106.11	107.37	46.348	97.323	88.058	47.570
12	*trans*-3-Hexenol	0.54810	0.31420	0.27530	0.29250	0.76610	0.24750	0.18120	0.05410	0.15580	0.21680	0.38520	0.06480
13	*cis*-3-Hexenol	0.86090	1.8575	0.81970	3.0739	2.2557	1.1672	7.6407	0.94050	1.1824	5.1993	2.2880	0.65320
14	*trans*-2-Hexenol	0.87680	0.19670	0.15950	0.08160	0.23410	0.09040	0.39450	0.15020	0.26340	0.14690	0.18210	0.10460
	Total hexenols (12–14)	2.2858	2.3684	1.2545	3.4480	3.2559	1.5051	8.2164	1.1448	1.6016	5.5630	2.8553	0.82260
15	2-Phenylethanol	6.2140	7.7754	10.168	4.8268	6.7169	10.086	5.8871	11.786	8.4133	6.1256	7.6716	5.0581
16	2-Methylpropanoic acid	12.605	1.7855	3.9440	0.09410	0.89850	3.7076	0.08160	1.0096	2.8233	0.47600	3.2056	1.3925
17	3-Methylbutanoic acid	1.1343	2.3928	5.6561	0.30930	1.8304	5.7983	0.43540	1.2861	4.5956	2.5342	1.2666	1.6542
	Total isoacids (16–17)	13.739	4.1783	9.6001	0.40340	2.7289	9.5059	0.51700	2.2957	7.4189	3.0102	4.4722	3.0467
18	Hexanoic acid	1.6201	4.9763	9.4946	1.0597	3.6413	8.3410	1.3366	5.2556	5.2255	1.8638	3.8292	3.7873
19	Octanoic acid	0.85750	3.1547	3.1684	1.4349	1.6071	3.0439	1.0973	1.6518	2.3937	0.94170	1.5288	1.0455
20	Decanoic acid	13.149	5.0881	4.8150	8.2041	3.1179	1.3512	0.39330	1.2817	1.1143	6.2853	5.3954	0.14400
21	Dodecanoic acid	1.2076	0.60800	1.1218	0.23210	0.22200	0.40160	0.71380	0.73140	0.22230	0.29960	0.41840	0.15440
	Total carboxylic acids (18–21)	16.834	13.827	18.600	10.931	8.5883	13.138	3.5410	8.9205	8.9558	9.3904	11.172	5.1312
22	Ethyl acetate	7684.4	1185.7	327.19	6686.3	1980.2	1139.7	7184.3	1422.8	135.26	6650.1	3155.3	806.45
23	Methyl acetate	714.44	78.045	36.663	570.97	155.38	109.79	712.49	136.15	14.258	620.93	303.37	92.710
24	3-Methylbutyl acetate	30.770	5.7958	1.2498	25.392	8.8323	1.5458	28.580	13.103	4.3578	29.322	13.144	3.3257
25	Hexyl acetate	12.146	2.4754	0.71140	7.9240	3.5995	0.59430	8.7340	4.5850	1.6683	9.1876	5.4027	1.2035
26	2-Phenylethyl acetate	0.54640	0.37160	0.10230	0.17430	0.18190	0.05560	0.19020	0.03670	0.05270	8.2865	0.35250	0.17850
	Total acetates (24–26)	43.462	8.6428	2.0635	33.490	12.614	2.1957	37.504	17.724	6.0788	46.796	18.899	4.7077
27	Ethyl butyrate	0.90940	0.13650	0.04210	0.86320	0.18490	0.07790	0.81590	0.22750	0.12170	0.77070	0.34030	0.10800
28	Ethyl hexanoate	19.102	5.5353	1.7745	15.790	7.7814	1.9448	14.013	11.945	3.6952	20.235	10.258	2.7454
29	Ethyl octanoate	10.829	6.5391	3.4678	6.9108	6.5494	3.4831	11.811	8.3759	3.6181	7.8429	6.2599	2.2741
30	Ethyl decanoate	7.8339	4.3291	2.3227	3.9562	4.1138	2.0079	9.0973	6.2527	1.7779	5.3241	4.4812	1.4473
31	Ethyl dodecanoate	5.4385	2.9850	1.5768	3.7395	2.6786	1.1544	3.8906	1.9512	0.83150	2.3601	2.3112	0.43470
	Total esters (27–31)	44.112	19.525	9.1839	31.259	21.308	8.6681	39.627	28.752	10.044	36.533	23.651	7.0095
32	Diethyl butanoate	9.1472	27.680	36.621	8.1082	23.500	29.676	5.2980	15.297	19.370	11.993	29.384	19.471
33	Ethyl 2-hydroxypropanoate	149.21	482.61	646.60	156.45	359.56	514.61	162.59	335.75	599.99	224.44	500.02	663.49

[a] Grouping of compounds within each family follows accepted methods based on role upon aroma

Table 3 Analysis of variance on the concentration of volatiles obtained for Alvarinho. Values in bold are statistically significant at the 5% level[a]

		Methanol	Ethanal	Acetal	Hexanol	*trans*-3-Hexenol	*cis*-3-Hexenol	*trans*-2-Hexenol	2-Phenylethyl acetate	Ethyl acetate	Methyl acetate	Diethyl butanoate	Ethyl-2-hydroxypropanoate	Sum of alcohols	Sum of isoacids	Sum of carboxylic acids	Sum of acetates	Sum of esters
F ratio	*t*	15.027	3.289	3.726	34.261	0.798	5.921	2.593	7.830	7.055	12.392	6.578	5.824	21.082	7.691	11.840	2.135	2.949
	f	71.028	8.813	16.420	4.937	2.474	4.973	2.395	23.145	17.222	12.215	2.970	7.175	813.097	23.213	0.375	328.519	137.926
	*t*f*	2.639	2.340	2.355	1.753	0.513	1.821	2.984	9.433	1.670	0.0676	2.145	0.739	4.995	13.064	2.001	5.359	2.325
P value	*t*	**<0.0001**	**0.0315**	**0.0197**	**<0.0001**	0.5031	**0.0022**	0.0676	**0.0004**	**0.0008**	**<0.0001**	**0.0012**	**0.0024**	**<0.0001**	**0.0004**	**<0.0001**	0.1128	**0.0457**
	f	**<0.0001**	**0.0008**	**<0.0001**	**0.0127**	0.0984	**0.0124**	0.1056	**<0.0001**	**<0.0001**	**<0.0001**	**0.0640**	**0.0024**	**<0.0001**	**<0.0001**	0.6898	**<0.0001**	**<0.0001**
	*t*f*	0.0318	0.0520	0.0507	0.1369	0.7941	0.1225	0.0181	**<0.0001**	0.1568	0.6700	0.0178	0.6214	**0.0008**	**<0.0001**	0.0912	**0.0005**	0.0533

[a] *F* ratio, ratio of mean sum of squares; *P* value, probability that such a high value of the *F* ratio arises from pure chance; *t* fermentation time, *f* fraction of distillate; *t*f* interaction between *t* and *f*

Table 4 Analysis of variance on the concentration of volatiles obtained for Loureiro. Values in bold are statistically significant at the 5% level[a]

		Methanol	Ethanal	Acetal	Hexanol	*trans*-3-Hexenol	*cis*-3-Hexenol	*trans*-2-Hexenol	2-Phenylethyl acetate	Ethyl acetate	Methyl acetate	Diethyl butanoate	Ethyl-2-hydroxypropanoate	Sum of alcohols	Sum of isoacids	Sum of carboxylic acids	Sum of acetates	Sum of esters
F ratio	*t*	6.901	5.068	4.673	9.963	1.556	12.537	5.447	7.830	1.882	2.165	16.948	3.858	21.406	1.026	5.216	14.273	6.739
	f	26.233	27.765	136.741	292.158	1.016	59.749	6.071	23.145	399.857	331.366	96.803	81.381	76.445	0.452	1.437	12.061	4.465
	*t*f*	1.688	0.0307	11.711	3.102	0.841	17.392	4.741	9.433	4.309	5.684	7.053	0.803	5.426	2.045	1.486	0.667	2.496
P value	*t*	**0.0009**	**0.0050**	**0.0074**	**<0.0001**	0.2169	**<0.0001**	**0.0034**	**0.0004**	0.1501	0.1091	**<0.0001**	**0.0172**	**<0.0001**	0.3924	**0.0102**	**<0.0001**	**0.0010**
	f	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.3722	**<0.0001**	0.0053	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.6400	0.2480	**<0.0001**	**0.0185**
	*t*f*	0.1521	**0.0307**	**<0.0001**	**0.0149**	0.5466	**<0.0001**	**0.0012**	**<0.0001**	**0.0023**	**0.0003**	**<0.0001**	0.5738	**0.0004**	0.0848	0.2106	0.6768	**0.0402**

[a] *F* ratio, ratio of mean sum of square; *P* value, probability that such a high value of the *F* ratio arises from pure chance; *t* fermentation time; *f* fraction of distillate; *t*f* interaction between *t* and *f*

Table 5 Probability associated with Fisher's protected least significant difference tests on concentrations of volatiles obtained for Alvarinho for various pairwise combinations of the manipulated variables: fermentation time, *t*, and fraction of distillate, *f*. The values in bold are statistically significant at the 5% level

t	Methanol	Ethanal	Acetal	Hexanol	*trans*-3-Hexenol	*cis*-3-Hexenol	*trans*-2-Hexenol	2-Phenylethyl acetate	Ethyl acetate	Methyl-acetate	Diethyl butanoate	Ethyl-2-hydroxypropanoate	Sum of higher alcohols	Sum of isoacids	Sum of carboxylic acids	Sum of acetates	Sum of esters
3, 6	**<0.0001**	**0.0044**	**0.0032**	**0.001**	0.1412	**0.4376**	**0.0301**	0.1195	0.0546	0.3957	0.1411	0.5132	**0.0001**	**0.0018**	**0.0006**	0.9901	0.0648
3, 9	0.0546	0.0721	**0.0175**	**<0.0001**	0.2943	**0.0018**	0.8857	0.2316	0.5333	0.0065	0.2212	0.6377	0.5375	0.2918	**<0.0001**	0.6524	0.3630
3, 12	0.0831	0.2977	0.1018	**<0.0001**	0.4149	**0.0024**	0.9059	**0.0019**	**0.0144**	**<0.0001**	**0.0001**	**0.0027**	**<0.0001**	**0.0002**	**<0.0001**	**0.0360**	0.3609
6, 9	**0.0129**	0.2440	0.5047	**<0.0001**	0.6626	**0.0138**	**0.0215**	0.0079	**0.0129**	**0.0006**	0.7965	0.2637	**0.0008**	**0.0268**	0.2435	0.6614	**0.0076**
6, 12	**<0.0001**	0.0553	0.1462	**<0.0001**	0.5012	0.0179	**0.0393**	0.0887	**<0.0001**	**<0.0001**	**0.0083**	**0.0004**	**0.0121**	0.4126	0.1238	**0.0370**	0.3338
9, 12	**0.0006**	0.4312	0.4225	0.8666	0.8121	0.9149	0.7934	**<0.0001**	0.0601	0.1195	**0.0042**	**0.0093**	**<0.0001**	**0.0034**	0.6988	0.0932	0.0730
f																	
Cab, Cor	**<0.0001**	**0.0021**	**<0.0001**	0.2725	0.0918	**0.3655**	0.0518	**<0.0001**	**<0.0001**	**0.0002**	0.0681	**0.0267**	**<0.0001**	**<0.0001**	0.4171	**<0.0001**	**<0.0001**
Cab, Cau	**<0.0001**	**0.0004**	**<0.0001**	**0.0037**	**0.0452**	**0.0040**	0.0882	**<0.0001**	**<0.0001**	**0.0001**	0.6902	**0.0006**	**<0.0001**	**<0.0001**	0.8653	**<0.0001**	**<0.0001**
Cor, Cau	0.2499	0.5729	0.9497	0.0545	0.7340	**0.0379**	0.7969	0.9029	0.6906	0.8618	**0.0285**	0.1572	**<0.0001**	0.8922	0.5198	**<0.0001**	**<0.0001**

Table 6 Probability associated with Fisher's protected least significant difference tests on concentrations of volatiles obtained for Loureiro for various pairwise combinations of the manipulated variables: fermentation time, *t*, and fraction of distillate, *f*. The values in bold are statistically significant at the 5% level

t	Methanol	Ethanal	Acetal	Hexanol	*trans*-3-Hexenol	*cis*-3-Hexenol	*trans*-2-Hexenol	2-Phenylethyl acetate	Ethyl acetate	Methyl-acetate	Diethyl butanoate	Ethyl 2-hydroxypropanoate	Sum of higher alcohols	Sum of isoacids	Sum of carboxylic acids	Sum of acetates	Sum of esters
3, 6	**0.0156**	**0.0056**	**0.0011**	0.9650	0.7262	**0.0084**	**0.0012**	0.1195	0.4701	0.9348	**0.0153**	**0.0427**	**<0.0001**	0.3834	0.0907	0.3296	**0.0195**
3, 9	0.6959	**0.0013**	**0.0210**	**0.0008**	0.1268	**<0.0001**	0.0791	0.2316	0.5888	0.6934	**<0.0001**	0.1355	**0.0006**	0.8730	0.9677	**0.0214**	**0.0001**
3, 12	0.0538	**0.0050**	**0.0090**	0.1194	0.3308	**0.0001**	**0.0017**	**0.0019**	0.0985	**0.0334**	**0.0122**	0.3592	**0.0183**	0.1280	0.2821	**<0.0001**	0.2283
6, 9	**0.0389**	0.5829	0.2647	**0.0007**	0.0633	**0.0040**	0.0958	0.0079	0.2103	0.7546	**<0.0001**	0.5695	**0.0002**	0.4753	0.0982	**0.0017**	0.0713
6, 12	**<0.0001**	0.9652	0.4365	0.1297	0.1890	0.1302	0.9078	0.0887	0.3405	**0.0401**	0.9278	**0.0045**	**<0.0001**	0.5038	0.5220	**<0.0001**	0.2304
9, 12	**0.0223**	0.6131	0.7312	**<0.0001**	0.5673	0.1348	0.1198	**<0.0001**	**0.0313**	0.0779	**0.0001**	**0.0190**	0.2128	0.1711	0.3002	**0.0461**	**0.0040**
f																	
Cab, Cor	**0.0942**	**<0.0001**	**<0.0001**	**<0.0001**	0.6135	**<0.0001**	**0.0101**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.3943	**0.0051**	**<0.0001**	0.4405
Cab, Cau	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.3751	**<0.0001**	**0.0025**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.9334	0.6778	**<0.0001**	**0.0065**
Cor, Cau	**<0.0001**	0.7685	**0.0020**	**<0.0001**	0.1678	**0.0068**	0.5960	0.9029	**<0.0001**	**0.0001**	0.1011	**<0.0001**	0.8699	0.4417	**0.0147**	0.7118	**0.0420**

significance. As a rule, the effect of *t* is statistically significant at a higher level of significance than that of *f*, whereas, as expected, the interaction $t*f$ is responsible only for a minor effect.

Inspection of Tables 5 and 6 shows that, depending on the group of volatiles considered, the effect of fermentation time period (consecutive or alternated) may or may not be statistically significant at the 5% level. In terms of the effect of the distillation cut, there is no statistical difference between the composition of any fraction in terms of the sum of the concentrations of 3-methylbutanoic and 2-methylpropanoic acids (Loureiro), while for Alvarinho the same is observed in terms of the sum of the concentrations of the acetates. In general, depending on its intrinsic volatility, the effect of the distillation cut upon the concentration of a given compound may or may not be statistically significant, e.g., ethanal is typically a head product (owing to its high volatility, it is mostly recovered in the first fraction of distillate) and this fact could explain the absence of a statistical difference between the heart and tail fractions in terms of the concentration of this component.

PCA data

The results of the PCA are depicted in Figs. 3 and 4 for the measured variables (composition of 15 volatiles)

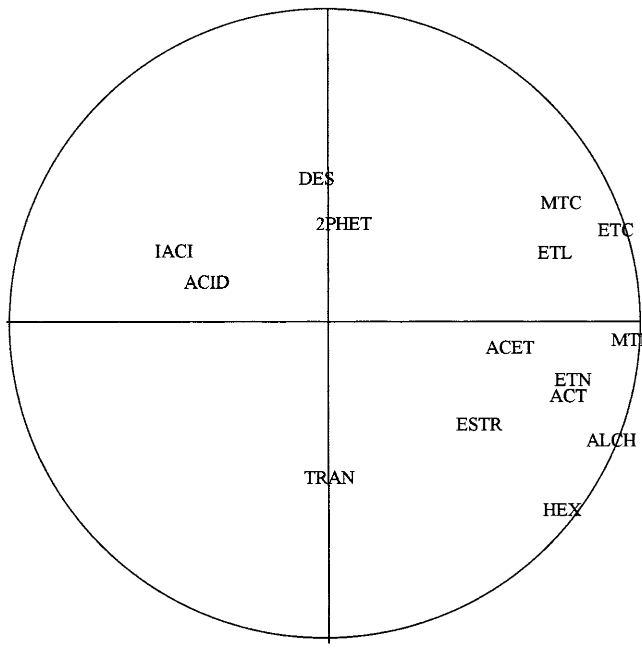

Fig. 3 Principal component plot of contents of bagaceira obtained from Alvarinho in terms of volatiles. *ETL* ethyl 2-hydroxypropanoate; *DES* diethyl butanoate; *TRAN* sum of *cis*-3-hexenol + *trans*-3-hexenol + *trans*-2-hexenol; *ACID* sum of carboxylic acids; *IACI* sum of 2-methylpropanoic acid + 3-methylbutanoic acids; *2PHET* 2-phenylethanol; *ACT* 1,1-diethoxyethane; *MET* methanol; *ALCH* sum of higher alcohols; *ESTR* sum of esters of carboxylic acids; *ACET* sum of acetates; *MTC* methyl acetate; *ETC* ethyl acetate; *ETN* ethanal

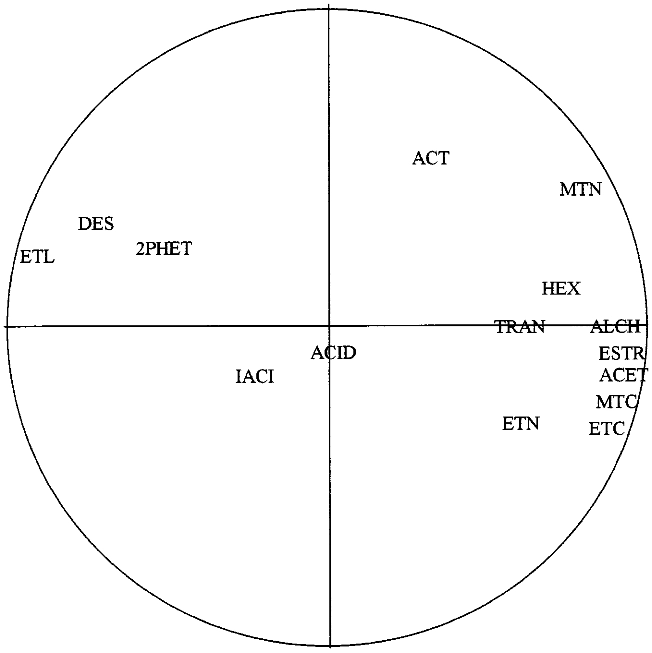

Fig. 4 Principal component plot of contents of bagaceiras obtained from Loureiro in terms of volatiles. Abbreviations as Fig. 3

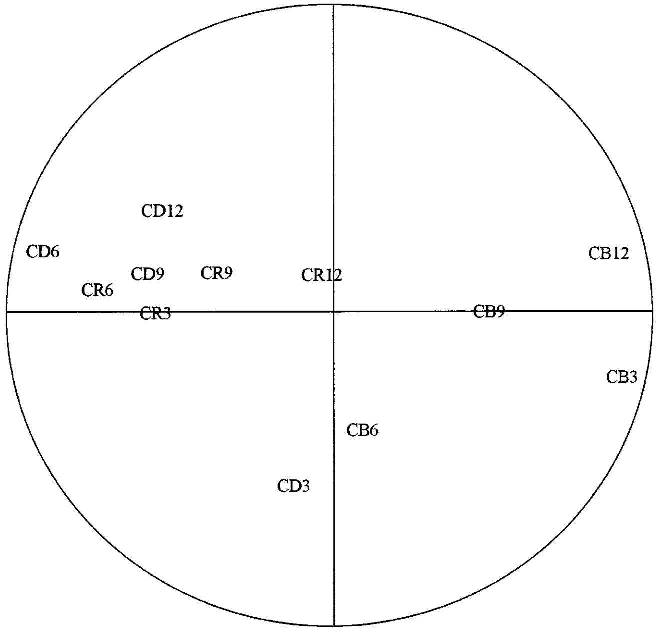

Fig. 5 Principal component plot of technological manipulated parameters of bagaceiras from Alvarinho in terms of overall composition. *CB* fraction of cabeças; *CR* fraction of coração; *CD* fraction of caudas; *3, 6, 9, 12* fermentation time (weeks)

and in Figs. 5 and 6 for the manipulated variables (fermentation time and distillate fraction), for Alvarinho and Loureiro, respectively.

Inspection of these figures indicates that the percentage of variance explained by the first two principal components is 95.9% and 98.9% for Loureiro and Alvarinho, respectively; in these cases, the third principal component explains as little as 3% and 1%, respectively, of the total variance, and no significant correlations exist between this axis and any of the variables. For Loureiro, the sums of the concentrations of alcohols, of esters, of acetates, of hexenols, and of isoacids, as well as the individual concentrations of methyl acetate, ethyl acetate, and ethanal, are positively correlated with the first principal component, whereas the individual concentrations of ethyl 2-hydroxypropanoate, diethyl butanoate, and 2-phenylethanol are negatively correlated with this axis. For Alvarinho, all variables are correlated with the first principal component, except for the individual concentrations of diethyl butanoate and 2-phenylethanol, and the sum of the concentrations of hexenols, which are correlated with the second principal component. It is interesting to note that, for Loureiro, the fractions of cabeças, coração, and caudas are associated in clusters that are well defined and move in the negative direction of the first principal component in this order, whereas increasing the time of fermentation makes the clusters move upwards in the direction of the second principal component. For Alvarinho, there is a clear difference between cabeças and the other fractions, although the coração and caudas fractions are intermixed with respect to time of fermentation upon increase; this latter variable makes the clusters move in the upward direction of the second principal component, especially in the case of the caudas fraction.

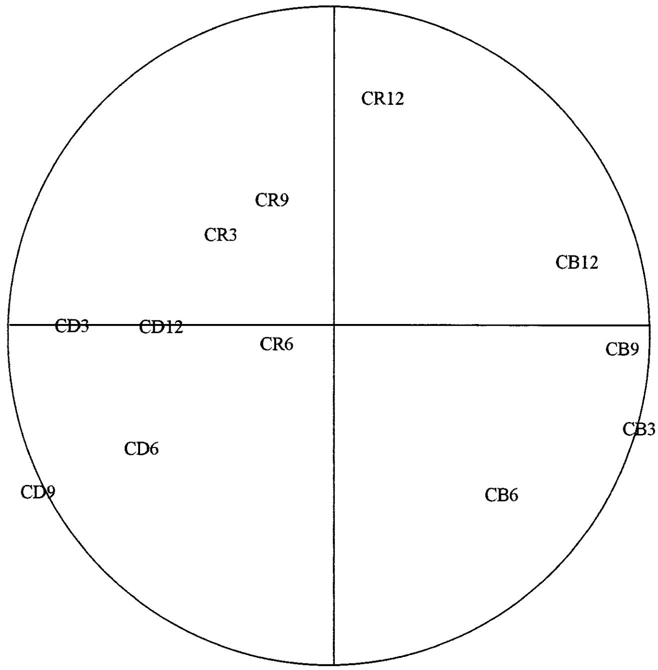

Fig. 6 Principal component plot of technological manipulated parameters of bagaceiras from Loureiro in terms of overall composition. Abbreviations as Fig. 5

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