Volatile Contents of Grape Marcs in Portugal

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The present study reports on the composition of volatiles of most grape marcs (*bagaceiras*) commercially available in Portugal. These spirits, which are a valuable by-product of the winemaking industry, are obtained via steam-distillation of grape pomace after storage under anaerobic conditions for a given period of time. An impetus for this research is the increasingly strict European legal standards pertaining to the levels of health hazard volatile compounds (e.g., methanol) and the lack of comprehensive studies on this topic for Portuguese *bagaceiras*. Assays were performed by gas/liquid chromatography for alcohols (methanol, 2-butanol, 1-propanol, 2-methyl-propanol, allylic alcohol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, hexanol, *trans*-3-hexenol, *cis*-3-hexenol, *trans*-2-hexenol, and 2-phenyl-ethanol), carboxylic acids (isobutyric, isovaleric, hexanoic, octanoic, decanoic, and dodecanoic acids), esters (ethyl acetate, ethyl butyrate, isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl succinate, 2-phenyl-ethyl-acetate, and ethyl dodecanoate), as well as for an aldehyde (ethanal) and its acetal (diethoxy-1-1-ethane). The average values obtained for Portuguese *bagaceiras* (and the corresponding ranges) were comparable with corresponding values for grape pomaces manufactured in other Mediterranean countries (viz. Spain). The mean contents of methanol were ca. 3389 mg/liter, a value below the accepted threshold of 5000 mg/liter for a 50% (v/v) ethanol-containing grape marc (EC Regulation No. 1576/89).

INTRODUCTION

The grape pomace which results from crushing the grapes during the winemaking process is in principle a low value agricultural by-product. However, farmers in Mediterranean countries often store such pomace in closed containers for several days so as to promote spontaneous anaerobic fermentation of the sugar residues. Such fermentations are effected by the native microflora on the grape pomace and lead to the production of a variety of volatiles which play an important role in flavor generation. Following this storage period, the pomace is manually transferred to copper batch stills, and steam distilled to release the volatiles. The first fraction of the condensate (termed heads or *cabeças*) is, in part, disposed off or redistilled due to its excessively high content of methanol. The following condensate (termed heart or *coração*) is a complex mixture, where most compounds are responsible for unique flavors; this intermediate fraction is sold as *bagaceira* proper. Due to its composition in terms of heavy components, the final fraction (termed tails or *caudas*) is disposed off or redistilled together with poor marcs. In the batch steam-operated stills, the first step of

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steam distillation may be complemented with a batch distillation termed rectification; this second process is common practice in the "Região dos Vinhos Verdes" where *Charentais*-type apparatuses are often employed.

Although bagaceiras are highly appreciated marcs especially after gourmet meals, their *per capita* annual consumption in Portugal (ca. 1.8 kg) is one of the lowest in Europe (Cascão, 1989). Exports of these specialty drinks have been in recent years more and more hampered by the increasingly strict food quality standards prevailing in most European countries (EC Regulation No. 1576/89), whereas internal consumption has been hampered by the even more restrictive Portuguese law. Due to a lack of detailed knowledge on the volatiles of bagaceiras commercially available, prejudiced arguments rather than rational queries may be raised upon such traditional Portuguese spirits. This research work has attempted, based on incipient knowledge pertaining to Portuguese bagaceiras, to generate a consistent and comprehensive database on the chemical composition of such spirits in order to help backup health hazard and food quality assessments.

MATERIALS AND METHODS

Samples

Eleven commercial bagaceiras, namely Adega Cooperativa de Borba, Aldeia Velha, Aveleda, Barrocão, CR&F, Croft, Genuína Barrocão, Caves do Barrocão, Quinta da Senhora do Monte, Quinta do Estanho, Valegrande, and Teobar, were randomly bought at local markets between October 1993 and January 1994. Samples of these Portuguese grape marcs (which originate from various regions) were investigated for volatiles as detailed below. For the sake of objectiveness, these bagaceiras were randomly assigned labels **BAG1** through **BAG11.**

Chemicals

Standards (chromatographic grade) of alcohols (methanol, 2-butanol, 1-propanol, 2-methyl-propanol, allylic alcohol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, hexanol, *trans*-3-hexenol, *cis*-3-hexenol, *trans*-2-hexenol, 2-phenyl-ethanol, 3-octanol, and 4-methyl-2-pentanol) and esters (ethyl acetate, ethyl butyrate, isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl succinate, 2-phenyl-ethyl-acetate, and ethyl dodecanoate) were purchased from Merck (Schuchardt, Switzerland). Standards of carboxylic acids (isobutyric, isovaleric, hexanoic, heptanoic, octanoic, decanoic, and dodecanoic acids), acetal (diethoxy-1-1-ethane), and ethanal (acetaldehyde) were purchased from Sigma (Sigma Chemical, U.S.A.). Ether, hexane, and sulfuric acid were obtained from Merck (Schuchardt, Switzerland).

Chromatographic Assays

For alcohol, acetal, ethyl acetate, and ethanal assays, a 5-ml sample of each bagaceira was mixed with 50 μl of an internal standard solution (50 g of 4-methyl-2-pentanol per liter of ethanol; Bertrand, 1988). The injector was maintained at 200°C and was operated in the split mode. Separation was achieved in a 50 m $\times$ 0.25 mm $\times$ 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 to 200°C at 3°C/min, and 200°C for 20 min. Detection was by flame ionization 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 in general present in relatively low concentrations) a 10-ml sample of each bagaceira was diluted with 40 ml of deionized water (in order to lower the total alcohol content to ca. 10% (v/v)), and the resultant solution was added with 2 ml of a 40 mg/liter solution of 3-octanol and 2 ml of a 90 mg/liter solution of heptanoic acid (used here as internal standards). 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 ether and hexane, and this procedure was repeated twice with 2 ml of the same solvent (Bertrand, 1975). The injector was maintained at 250°C and was operated in the splitless mode. Separation was achieved in a 25 m × 0.32 mm × 0.3 μm capillary column FFAP CB-Wcot fused silica (Chrompack, The Netherlands). 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 cases, aliquots of 0.5 μl were automatically injected into a gas/liquid chromatograph Autosystem (Perkin–Elmer, Norwalks, U.S.A.). The helium flow rate was 2 ml/min and the gases required by the flame ionization detector were supplied at pressures of 90 kPa (H_2) and 110 kPa (air). Integration was done using the software OMEGA (PE NELSON, Norwalks, U.S.A.), taking as reference the peak areas corresponding to the internal standards. Analyses were carried out in triplicate and their average was used in the results section.

Qualitative (and quantitative) analyses of the peaks in the samples analyzed were made by comparison of their retention times (or their areas) with those of the standards (or with those of the internal standards). In the calibration, the response factor of each compound of interest, RF_i, was calculated by $RF_i = \left(\frac{A_{is}}{A_{si}}\right) \cdot \left(\frac{C_{si}}{C_{is}}\right)$, where A_{is} and A_{si} are the peak areas of the chromatographic internal standard and of the chromatographic standard of the compound of interest, respectively, and where C_{is} and C_{si} are the molar concentrations of the chromatographic internal standard and of the chromatographic standard of the compound of interest, respectively. In the actual quantitation, the molar concentration of each compound of interest, C_i, was determined via $C_i = \left(\frac{A_i}{A_{is}}\right) \cdot C_{is} \cdot RF_i$, where A_i is the area of peak of interest.

For the sake of book keeping, the actual data obtained for each individual bagaceira were replaced in this report by the corresponding mean, standard deviation, and range of all bagaceiras for each compound assayed. Although the range is easily calculated by plain inspection of the dataset, the standard deviation is a more reliable measure of the spread of the data because it depends on the whole dataset rather than only two data points; however, both statistics were included for comparison purposes. The linear association between the concentrations of the various compounds assayed was tested via computation of the regression coefficient, and the decision on whether the correlation was significant was done on the 10% level (Caulcutt and Boddy, 1991).

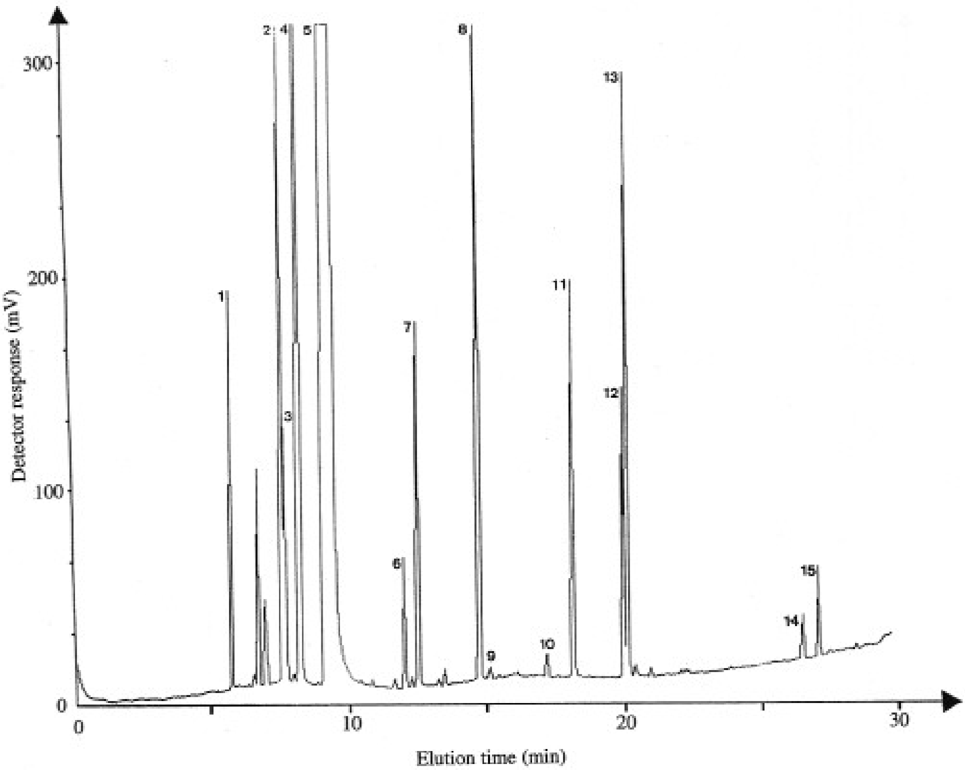

FIG. 1. Gas chromatogram obtained after direct injection of a typical bagaceira. 1, ethanal; 2, ethyl acetate; 3, acetal; 4, methanol; 5, ethanol; 6, 2-butanol; 7, 1-propanol; 8, 2-methyl-1-propanol; 9, allylic alcohol; 10, 1-butanol; 11, 4-methyl-2-pentanol (internal standard); 12, 2-methyl-1-butanol; 13, 3-methyl-1-butanol; 14, ethyl lactate; 15, hexanol.

RESULTS AND DISCUSSION

Alcohols are major products of fermentation of sugars carried out primarily by yeasts. Besides ethanol, a number of alcohols are present in distilled beverages; these compounds are either contributed by the grapes themselves or are formed as a result of microbial action during anaerobic fermentation. Free fatty acids are normal components of distilled alcoholic beverages and are mainly produced via yeast metabolism of carbohydrates. Fatty acids are related to a large group of aroma compounds which includes esters. Some of the most important esters in marcs are those of octanoic, decanoic, and dodecanoic acids. Aldehydes can be found in distilled alcoholic beverages and are thought to be an indicator of spontaneous oxidation or of the activity of unwanted contamination bacteria (Amerine, 1980). More than 90% of the total aldehyde content is accounted for by ethanal and its diethyl acetal.

Typical chromatograms of a Portuguese bagaceira are presented in Figs. 1 and 2. The quantitative results for the composition of the various alcohols and aldehyde, carboxylic acids, and esters are depicted in Table 1.

All samples exhibited ethanol contents ranging from 40 to 52.5% (v/v). Higher alcohols are by far the most abundant class of secondary constituents of grape marcs.

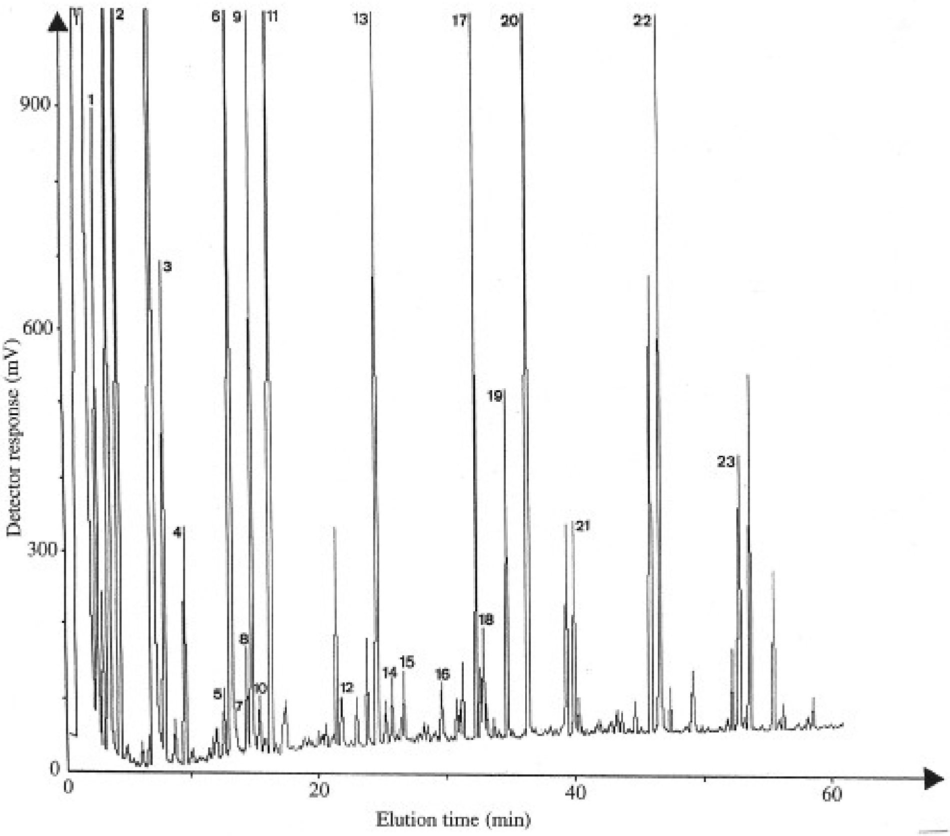

FIG. 2. Gas chromatogram obtained after injection of the ether/hexane extract of a typical bagaceira. 1, ethyl butyrate; 2, isoamyl acetate; 3, ethyl hexanoate; 4, hexyl acetate; 5, ethyl lactate; 6, hexanol; 7, *trans*-3-hexenol; 8, *cis*-3-hexenol; 9, 3-octanol (internal standard); 10, *trans*-2-hexenol; 11, ethyl octanoate; 12, isobutyric acid; 13, ethyl decanoate; 14, isovaleric acid; 15, diethyl succinate; 16, 2-phenyl-ethyl-acetate; 17, ethyl dodecanoate; 18, hexanoic acid; 19, 2-phenyl-ethanol; 20, heptanoic acid (internal standard); 21, octanoic acid; 22, decanoic acid; and 23, dodecanoic acid.

It is known that the level of amyl alcohols is a predictor of sensory character; amyl alcohols (viz. 3-methyl-1-butanol and 2-methyl-1-butanol) range from 600 to 1800 mg/liter, with an average of 1191.04 mg/liter over all bagaceiras analyzed. The lower levels of amyl alcohols indicate light-bodied grape marcs.

The EC regulation 1576/89 established general manufacturing procedures of marc distillates and fixed common analytical composition limits, i.e., 86% (v/v) of ethanol as the highest proof for the crude distillate and 37.5% (v/v) as the minimal proof at bottling, a maximum methanol content of 1000 mg/100 ml ethanol, and a maximum 2-butanol content of 30 mg/100 ml ethanol. The methanol and 2-butanol contents of most bagaceiras (see Table 1) are within the limits of acceptability of the aforementioned EEC regulation and those set forth by INDC (the Portuguese Institute of Consumer Defense). Exceptions to the permissible methanol upper limit found in our survey were 1530 mg methanol/100 ml ethanol and 1030 mg methanol/100 ml ethanol. These values may be the result of using red grape pomace as raw material and/or of

TABLE 1

ALCOHOLS, ALDEHYDES, CARBOXYLIC ACIDS, AND ESTERS OF PORTUGUESE BAGACEIRAS (MG/LITER)

Component	Mean	St. deviation	Range
ethanol % (v/v)	45	4.5	40.0-52.5
methanol	3400	1280	1590-6410
ethanal	600	320	200-1070
acetal	300	210	19-680
2-butanol	50	37	0.0-133
1-propanol	250	76	142-377
2-methyl-propanol	360	160	190-660
1-butanol	23	7.1	13.4-37.6
2-methyl-1-butanol	280	94	135-445
3-methyl-1-butanol	920	292	463-1360
TOTAL (2-butanol to 3-methyl-1-butanol)	1880	611	1100-3000
allylic alcohol	17	18.6	0.0-62
hexanol	60	19	36-95
trans-3-hexenol	1	3.7	0.02-12.6
cis-3-hexenol	2	2.1	0.43-3.15
trans-2-hexenol	0.3	0.31	0.0-0.95
2-phenyl-ethanol	10	3.5	5.9-17.4
isobutyric acid	2	2.7	0.4-9.8
isoamylic acid	3	1.7	0.9-5.3
hexanoic acid	2	1.0	0.9-4.0
octanoic acid	4	2.5	0.4-8.6
decanoic acid	4	2.3	0.4-7.5
dodecanoic acid	1	0.7	0.2-2.5
TOTAL (hexanoic to dodecanoic acids)	11	5.4	1.9-17.3
ethyl acetate	200	160	50-530
isoamyl acetate	6	4.4	1.1-16.4
hexyl acetate	0.3	0.18	0.06-0.65
2-phenyl-ethyl-acetate	0.3	0.21	0.06-0.65
TOTAL (isoamyl acetate to 2-phenyl-ethyl-acetate)	6	4.5	1.8-16.9
ethyl butyrate	1.2	0.40	0.44-1.58
ethyl hexanoate	4	2.9	1.6-10.4
ethyl octanoate	11	9.5	2.0-16.7
ethyl decanoate	11	6.2	3.5-23.9
ethyl dodecanoate	2.3	1.3	0.85-5.17
TOTAL (ethyl butyrate to ethyl dodecanoate)	30	19	9-73
diethyl succinate	5	3.5	1.50-14.6
ethyl lactate	190	140	40-510

poorly performed distillation procedures (e.g., high degree of incorporation of *cabeças*). Methanol (which may, upon ingestion, lead to blindness and eventually death) is generated via degradation catalyzed by native pectinases in grape pomace; the rate of such degradation is enhanced by the extent of maceration of the pomace; and the concentration of methanol in the final bagaceira increases as the splitting between *cabeças* and *coração* is made earlier. With respect to 2-butanol, this compound was found in all bagaceiras but one. In the cases where its value was above the detection threshold, the values ranged from 2.19 to 26.7 mg/100 ml ethanol, so the upper permissible limit (30 mg/100 ml ethanol) was not violated in all cases. There is some consensus that 2-butanol originates from bacterial action, probably upon 2,3-butanediol. The presence of 2-butanol is deleterious to quality especially due to the off-flavor associated with it.

Second to acetic acid, the most abundant acids in the bagaceiras analyzed are octanoic at an average level of 4.01 mg/liter, decanoic at 3.67 mg/liter, hexanoic at 1.82 mg/liter, and dodecanoic at 1.1 mg/liter. It can be seen that octanoic and decanoic acids are the most important components of this family, except in what concerns one of the bagaceiras tested. A wide range was found for the sum of concentrations of free fatty acids (1.9 to 15.5 mg/liter) in these commercial spirits.

The fatty acid ethyl ester contents of the bagaceiras studied ranged from 9.16 to 73.2 mg/liter, with an average of 29.4 mg/liter. Ethyl acetate, one of the most important esters due to its unpleasant flavor, ranged in content from 50.4 to 528 mg/liter, with an average of 210 mg/liter; these values can, in general, be considered to lie on the low side for spirits.

The sum of the concentration of ethanal and acetal was rather low for all bagaceiras, ranging from 220 to 1750 mg/liter, with an average of 900 mg/liter.

One traditional measure of flavor quality is the so-called Natural Flavor Component Index (NFCI) which is defined (Guymon, 1970) as the sum of the concentrations of all alcohols (except ethanol and methanol), all esters, and all free fatty acids. The highest among all samples displayed an NFCI of 3800 mg/liter, whereas the lowest possessed an NFCI of only 1400 mg/liter; these observations show a large diversity in terms of characteristics of commercial bagaceiras.

One further step in the analyses of volatiles may be taken by calculating the correlation coefficients (ρ) between the concentrations of each pair of volatile compounds at a time for all bagaceiras (coming from distinct regions and obtained from distinct feedstocks) considered together. The correlation coefficients are comprehensively listed in Table 2. High positive correlations between different components are an indication of either similar volatilities or products from the same microbial metabolic pathway. Significant positive correlations, equal to or higher than 0.9, which are well documented for other spirits (Versini, 1992), exist between 2- and 3-methyl-1-butanol, and between either of these two compounds and 2-methyl-1-propanol, and between ethyl hexanoate, ethyl octanoate, and ethyl decanoate. These compounds can be mainly considered as belonging to *coração*, so such high correlations observed can easily be explained by similar distillation properties. Other significant positive correlations exist between ethanal and its acetal (*cabeças*). In general, correlation between high 1-hexanol contents and high contents of *cis*-3, *trans*-3, and *trans*-2-hexenols appear to be typical of distillates from regions where grape pomaces are stored after winemaking without pH correction, as is the case for Portuguese bagaceiras. However, in our case, correlations between these components are not statistically significant. It is also

TABLE 2

Mutual Correlation Coefficients (ρ) for Linear Regression Analysis among the Various Compounds Analyzed

Comp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2		+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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Note. 1: ethyl acetate, 2: ethyl butyrate, 3: isoamyl acetate, 4: ethyl hexanoate, 5: hexyl acetate, 6: ethyl lactate, 7: ethyl octanoate, 8: ethyl decanoate, 9: diethyl succinate, 10: 2-phenyl-ethyl-acetate, 11: ethyl dodecanoate, 12: propionic acid, 13: isobutyric acid, 14: isoamylic acid, 15: hexanoic acid, 16: octanoic acid, 17: decanoic acid, 18: dodecanoic acid, 19: ethanal, 20: acetal, 21: methanol, 22: 2-butanol, 23: 1-propanol, 24: 2-methyl-propanol, 25: allylic alcohol, 26: 1-butanol, 27: 2-methyl-1-butanol, 28: 3-methyl-1-butanol, 29: hexanol, 30: *trans*-3-hexenol, 31: *cis*-3-hexenol, 32: *trans*-2-hexenol, and 33: 2-phenyl-ethanol.

+ : correlation coefficients higher than or equal to 0.9
- : correlation coefficients lower than 0.9

interesting to note that there is a very poor correlation between ethanal and ethyl acetate contents, although both products are mainly removed in *cabeças.* This observation suggests that ethanal is more likely a result of glucidic fermentation than of oxidative degradation of ethanol to acetic acid (Versini, *et al.*, 1991). The good correlation between 2-phenyl-ethyl-acetate and decanoic acid and between *trans*-2-hexenol and hexanoic acid can be explained once all of them are removed as *tail* products.

Comparing the results obtained with those obtained for *aguardiente Galicia* (Orriols *et al.*, 1991), a similar beverage originating from Spain (i.e., analogous raw material and distillation technique), it can be concluded that the major differences are those concerning acetal and ethyl acetate (typically *head* products), with higher contents in aguardiente (ca. 800 mg/liter for acetal and ca. 1300 mg/liter for ethyl acetate), and ethyl lactate (typically a *tail* product) with lower content in aguardiente (ca. 9 mg/liter). Hence, in can be concluded that aguardiente Galicia is richer in *head* products and poorer in *tail* products, but the methanol content is approximately the same for both aguardiente and bagaceira.

In general, it can be concluded that Portuguese bagaceiras are characterized by low levels of aldehydes and volatile esters, and a considerable variation in the levels of amyl alcohols. Most bagaceiras are of high quality and safe for human consumption in terms of methanol and 2-butanol contents, although this work indicates that in some cases improvement in the quality of the pomace subject to distillation is in order.

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