Relationships Between Storage Conditions of Grape Pomace and Volatile Composition of Spirits Obtained Therefrom

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This study reports on the effects of addition of tartaric acid, addition of pectinase, time of anaerobic storage, and the storage container material on the concentrations of volatiles in marc distillates of *Alvarinho* and *Loureiro* varieties. The aim of this work is to predict how the concentration of every major volatile responds to each manipulated processing factor in each of three distillate cuts, a condition necessary to rationalize the process of the manufacture of *bagaceiras*. Fusel alcohols were, quantitatively, the largest group of flavor compounds in all cases, ranging from 395 to 2029 mg/L. Ethyl acetate and ethyl lactate were the most concentrated esters, ranging from 176 to 9614 and from 0 to 310 mg/L, respectively. Fermentation time is the manipulated variable that most strongly affected composition of marcs in terms of higher alcohols, while addition of pectinases and material of container are the manipulated variables that most strongly affected composition in terms of methanol (with a concentration range of 2694 - 6960 mg/L) and 2-butanol (with a concentration range of 0 - 279 mg/L). The effect of pectinase addition is statistically the most significant for the methanol content, whereas the effect of fermentation time is statistically the most significant for the 2-butanol content.

KEY WORDS: grape pomace, *bagaceira*, distillation, methanol, marc

Bagaceiras are spirits obtained throughout Portugal via steam distillation of grape pomace after winemaking; however, those originating from *Região dos Vinhos Verdes* are particularly appreciated due to their unique bouquet. The grape pomace used to produce them is sometimes stored under poor environmental conditions for a relatively long time before distillation; in such cases, the resulting *bagaceiras* are of low quality, so their market value drops considerably. In the last decade, strongly flavored *bagaceiras*, possessing an *Appélation d'Origine Protegée* status, have been in great demand and *Comissão de Viticultura da Região dos Vinhos Verdes*, by appointment to the Portuguese Government, has enrolled in major programs of technical support action in attempts to make winemakers aware of the importance of preservation of pomace and in attempts to help them improve distillation techniques so that the resulting spirits possess a consistently high quality and are well suited for widespread sale and export. Furthermore, regulations imposed by the European Union (*viz.* Reg. 1576/89) have established general manufacturing procedures for all distilled spirits (including *bagaceiras*) and have, consequently, fixed the limits of concentration of several volatile compounds: *i.e.*, 86.0 (v/v) of ethanol as highest level for the raw distillate and 37.5% (v/v) as lowest level at bottling; 1% (w/v) of methanol (on ethanol basis) as upper limit and 0.14% (w/v) as lower limit for the sum of concentrations of higher alcohols (on ethanol basis, and expressing the concentrations of organic acids as acetic acid equivalent, those of esters as ethyl acetate equivalent, and those of aldehydes as acetaldehyde equivalent) [8].

The list of spirits similar to *bagaceiras* in terms of raw material, manufacturing techniques, and organoleptic properties encompasses "Orujo Gallego" (Spain), "Marc" (France), "Grappa" (Italy), and "Tsipouro" (Greece). In order to obtain all such spirits, pressed grapes after complete fermentation are employed, the composition of which depends on the actual storage conditions; such undesirable compounds as methanol and 2-butanol are formed during the first stages of storage, and so special care is to be exercised when manipulating storage variables.

Processing of pomace during storage takes advantage of spontaneous anaerobic fermentation in which bacteria convert water-soluble carbohydrates to lactic and acetic acids, which are in turn converted to alcohols, esters, carboxylic acids, and aldehydes by yeasts [12,13]. The inventory of soluble carbohydrates is sometimes increased via accelerated breakdown of such polysaccharides as pectins, which can be brought about by addition of pectinases. As a result of the fermentation process, the pH decreases, and the silage can be maintained for a relatively long time in the absence of extensive contamination leading to off-flavor production. An alternative technique commonly used in Italy consists of adding an acid (sulfuric, tartaric, or phosphoric) at the proportion of 1 L per 400 kg of solid pressed grapes [16] by spraying on the grape pomace prior to storage to reduce the pH and consequently limit the activity of bacteria, thus giving place to an

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adequate glucidic fermentation and preventing formation of methanol by inhibition of enzymes implicated in pectin degradation. During approximately the first week of storage, residual air is still present, and this enables aerobic multiplication of adventitious bacteria and yeasts to take place. After this initial period, the amount of secondary products generated depends directly on the duration of storage, which is determined in a completely empirical mode based on the degree of maturity of the grapes at the time of harvest, the temperature and relative humidity of the environment, and the availability of container space. Values reported elsewhere [15] indicate a 10% to 20% decrease in ethanol contents and a 15% increase in methanol as a consequence of improper conditions of storage. Furthermore, proliferation of some strains of yeasts and acetic acid bacteria can negatively affect the fermentation pattern via increase of the levels of acetic acid, generation of high levels of short chain fatty acids, and formation of 2-butanol, all of which account for off-flavors. Orriols [10] claimed that grape pomace stored under improper conditions produces distillates with high levels of methanol, 2-butanol, hexanol, acetic acid, ethanal, and acetal, as well as low levels of ethyl esters, thus leading to poor quality in terms of flavor and aroma. These conclusions can be extrapolated to Portuguese *bagaceiras* to some extent owing to the similarities of feedstock and processing conditions. Due to increasing downstream market demands, the average duration of the fermentation period has been deliberately decreasing and hence the fraction of unfermented pomace by the time of distillation has been gradually increasing. Finally, two types of storage containers have been used, *viz.* plastic containers with a leakage proof mobile lid or wooden containers covered with plastic bags and sand on top of them. Although the degree of anaerobiosis of the plastic containers is clearly greater than that of the wooden containers, the former are more expensive than the latter, and a balance is usually sought between these technological and economic factors.

The principal aim of distillation is to selectively extract volatile components present in grape pomace relative to other less volatile components usually associated with unpleasant flavors. With respect to distillation, two different techniques are followed: *(i)* direct distillation of fermented pomace, possibly added with lees and water immediately before distillation, or *(ii)* distillation of the aqueous liquid resulting from previous wash of the fermented pomace. The distillation process can be batch or continuous, but in both cases it is carried out slowly to avoid development of hot spots and consequent thermal degradation of the spirit. Since the distillate is very rich in methanol, a second distillation is often required (rectification) which lowers the methanol content down to the legal specifications, and almost completely eliminates acetaldehyde and low boiling esters (from methyl formate to ethyl propionate). When batch-wise operation is selected, sequential fractioning of distillate can alternatively serve the same purpose; in this case, two cuts along the distillation process are made, the first to separate the head products from the heart products, and the second to separate the heart products from the tail products, followed by mixing of the tail products to the head products which are subsequently subjected to a second independent distillation for non-food purposes. The head products, or the first fraction of condensate (also termed *cabeças*), is very rich in methanol; the following fraction (also termed *coração*) is a complex mixture of volatiles, most of which contribute organoleptic key notes; the tail products, or the last fraction (also termed *caudas*) is rich in less volatile compounds [8,12,13,14]. Definition of the points where the distillation cuts are to be made is done in an empirical fashion and is based only on the winemaker's experience and on the bulk concentration of alcohols as monitored by density; *viz.* the head products cut is usually made between 70% and 80% (v/v) and the tail products cut usually between 35% and 50% (v/v).

The aim of this research was to relate the concentration of the major esters, carboxylic acids, and aldehydes in each fraction of distillate (*cabeças, coração,* and *caudas*) to the level of addition of pectinases, the level of addition of tartaric acid, the storage time, and the material of the container, via postulation of empirical polynomial models and fitting of such models to experimental data generated according to a factorial design using two varieties of *Vinho Verde* white grapes. Although several experimental studies were conducted by Orriols [9,10], Soufleros [14], and Versini [17,18] with similar spirits, such studies were only concerned with distillation techniques and characteristics of equipment. This work attempts to complement existing knowledge on the distillation process with innovative assessments of the effects of the storage process on the characteristics of the final product.

Materials and Methods

Grape material: White grapes from *Alvarinho* variety were obtained from *Adega Cooperativa de Monção* (Monção, Portugal), whereas white grapes from *Loureiro* variety were obtained from *Direcção Regional de Agricultura de Entre Douro e Minho* (Vairão, Portugal). Grapes were harvested at similar maturation stages (*i.e.,* at a sugar content of 12 g and 14 g per 100 g of grape pomace for *Alvarinho* and *Loureiro*, respectively), and crushed and pressed in the usual fashion for the manufacture of white wine (*i.e.,* using a 20-ton horizontal press for two hours in four successive cycles). For each variety, grape pomace from a given batch was utilized for all experiments. The pH of the grape pomace was 3.4 and 3.6 for *Alvarinho* and *Loureiro*, respectively. Plastic containers (*Alvarinho*) and/or wooden containers (*Loureiro*) were filled with *ca.* 150 kg of grape pomace and covered to ensure anaerobic conditions.

Experimental design: The study encompassing *Alvarinho* was arranged in a 2^3 factorial design replicated twice with two levels of addition of tartaric acid, **T** (0 and 80 g per 100 kg of grape pomace), two storage times under anaerobic conditions, **t** (3 and 6 wk), and two levels of addition of pectinase, **E** (0 and 2 g/100 kg of grape pomace). The study encompassing *Loureiro* was arranged as a half fraction of a 2^4 factorial design replicated twice with two levels of addition of tartaric acid (0 and 80 g/100 kg of grape pomace), two times of storage under anaerobic conditions (3 and 5 wk), two levels of addition of pectinase (0 and 2 g/100 kg of grape pomace), and two types of container (plastic and wood), coupled with an extra set of two experiments (replicated twice) run at the center conditions (40 g tartaric acid and 1 g pectinase/100 kg of grape pomace, and anaerobic maturation for 4 wk), one using a plastic container and the other using a wooden container. The experimental layout is depicted in Tables 1 and 2 for *Alvarinho* and *Loureiro*, respectively. The pectinase utilized (ULTRAZYM®100G, from NOVO Nordisk, Bagsvaerd, Denmark) and the tartaric acid (commercial grade) were independently sprayed onto the pomace in the form of concentrated aqueous solutions (20 g/L) so as to obtain the aforementioned final ratios of addition per weight of pomace.

Distillation: The 150 kg-batches of fermented pomace of the grape varieties considered were steam distilled in copper batch stills after anaerobic storage for three and six weeks (*Alvarinho*) or three, four, and five weeks (*Loureiro*), using burning wood and natural gas, respectively, as heating source. Distillation products were collected as three fractions: *cabeças* (overall volume, V = 2 L), *coração* (V = 3.5 L), and *caudas* (V = 2 L), which correspond to variations of the volumetric percent of alcohol of 60% to 90% (v/v), 30% to 60% (v/v), and less than 30% (v/v). Distillates were collected in dark bottles, stoppered, and stored at 4ºC until analysis.

Chemical analyses: *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, isoamylic, hexanoic, heptanoic, octanoic, decanoic, and dodecanoic acids), acetal (diethoxy-1-1-ethane), and ethanal (acetaldehyde) were purchased from Sigma (St. Louis, MO). Ether, hexane, and sulfuric acid were obtained from Merck.

Chromatography: For assays of alcohols, acetal, ethyl acetate, and ethanal (which are in general present at relatively high concentrations), a 5-mL sample of each distillate fraction was mixed with 50 µL of an internal standard solution of 50 g of 4-methyl-2-pentanol per L of ethanol [4]. The injector of the gas chromatograph (Autosystem GC, from Perkin Elmer, Norwalk PA) 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 five minutes, a linear ramp from 40°C to 200°C at 3°C/minute, and 200°C for 20 minutes. 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 at relatively low concentrations), a 10-mL sample of each distillate fraction was diluted with 40 mL of deionized water in order to lower the total alcohol content to below 10% (v/v), and the resultant solution was added with 2 mL of a 40 mg/L solution of 3-octanol and 2 mL of a 90 mg/L solution of heptanoic acid (both used here as internal standards). The pH was adjusted to 2.0 using a few drops of concentrated sulfuric acid. The solution thus obtained was extracted for five minutes with 4 mL of a 50% (v/v) mixture of ether and hexane; this procedure was repeated twice with 2 mL of the same solvent [3]. The injector of the gas chromatograph was maintained at 250°C and was 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 Netherlands). The oven temperature program was as follows: 40°C for 5 minutes, a linear ramp from 40°C to 220°C at 3°C/minute, and 220°C for 20 minutes. 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, 0.5-µL aliquots were injected into the gas chromatograph using its autosampler. The flow rate of the carrier gas was 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 the software OMEGA™ (P. E. Nelson, Norwalk PA) taking as reference the peak areas corresponding to the internal standards. Chromatographic runs were carried out in triplicate, and their average was used as a single data point in the **Results** section; the average coefficient of variation for the triplicate assays ranged from 1.1% for octanoic acid to 7.1% for ethyl butyrate.

Modelling: The model to be fitted to the data regarding composition of each distillate fraction generated for *Alvarinho* in every experimental combination of manipulated variables was postulated in a completely empirical form and read: $\hat{y} = \bar{y} + a_1x_1 + a_2x_2 + a_3x_3 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3$, where $\hat{y}$ is the fitted response variable, $\bar{y}$ is the grand average of the overall dataset, a_1, a_2, and a_3 are the independent effects of addition of tartaric acid, time of fermentation, and addition of pectinase, respectively, a_{12}, a_{13}, a_{23}, and a_{123} are the interaction effects between the aforementioned manipulated variables, and x_1, x_2, and x_3 are coded, normalized variables defined as $x_1 = (T-40)/40$ (where T is the level of addition of tartaric acid, expressed in g/100 kg of grape pomace), $x_2 = (t-31.5)/10.5$ (where t is the time of grape storage, expressed in d), and $x_3 = (E-1)$ (where E is the level of addition of pectinase, expressed in g/100 kg of grape pomace).

Table 1. Experimental design followed with *Alvarinho* grape pomace.

	Effect		
R	1	2	3
1	+1	+1	+1
2	+1	+1	-1
3	+1	-1	+1
4	+1	-1	-1
5	-1	+1	+1
6	-1	+1	-1
7	-1	-1	+1
8	-1	-1	-1

Note: R- run; **1**- normalized level of addition of tartaric acid, defined as (T-40)/40; **2**- normalized time of fermentation, defined as (t-31.5)/10.5; **3**- normalized level of pectinase addition, defined as (E-1).

The model to be fitted to the data regarding composition of every distillate fraction generated for *Loureiro* in each experimental combination of manipulated variables was postulated in a completely empirical fashion and read: $\hat{y} = \bar{y} + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3$, where $\hat{y}$ is the fitted response variable, $\bar{y}$ is the grand average of the overall dataset, a_1, a_2, a_3 and a_4 are the independent effects of addition of tartaric acid, time of fermentation, addition of pectinase, and type of container, respectively, a_{12}, a_{13}, and a_{23} are the interaction effects between the aforementioned manipulated variables, and x_1, x_2, x_3, and x_4 are coded, normalized variables defined as $x_1 = (T - 40)/40$ (where T is the level of addition of tartaric acid, expressed in g per 100 kg of grape pomace), $x_2 = (t - 28)/7$ (where t is the time of grape storage, expressed in d), $x_3 = (E - 1)$ (where E is the level of addition of pectinase, expressed in g per 100 kg of grape pomace), and $x_4 = C$ (where C is the type of container; -1: plastic, +1: wood).

Table 2. Experimental design followed with *Loureiro* grape pomace.

	Effect			
R	1 (+234)	2 (+134)	3 (+124)	4 (+123)
1	+1	+1	+1	+1
2	+1	+1	-1	-1
3	+1	-1	+1	-1
4	+1	-1	-1	+1
5	-1	+1	+1	-1
6	-1	+1	-1	+1
7	-1	-1	+1	+1
8	-1	-1	-1	-1
9	0	0	0	-1
10	0	0	0	+1

Note: R- run; **1**- normalized level of addition of tartaric acid, defined as (T-40)/40; **2**- normalized time of fermentation, defined as (t-28)/7; **3**- normalized level of pectinase addition, defined as (E-1); **4**- type of container (-1, plastic; +1, wood).

+1 and -1 indicate, respectively, the addition or non-addition of 80 g tartaric acid/100 kg grape pomace (column 1); 6 and 3 weeks of storage (column 2); addition or non-addition of 2 g pectinase/100 g grape pomace (column 3).

In columns 1, 2, and 3: 0 indicates center conditions (40 g tartaric acid/ 100 kg grape pomace, 4 weeks of storage, and 1 g/100 g grape pomace), respectively.

In both cases, the models considered took the simplest form that can be postulated (*i.e.*, polynomial) and possessed the highest number of parameters that can be statistically fitted in view of the experimental designs chosen [*i.e.*, 8]. Estimates of the effects of manipulated variables and combinations thereof were obtained by linear regression of the aforementioned models to the experimental data produced. This procedure, based on minimization of the squares of the residuals, is valid if the experimental errors are normally distributed and if they have a constant variance [5], traits that were concluded to be correct after *a posteriori* diagnostics of residuals (not shown). The standard deviations, or square root of the sum of squares of residuals of every set of replicates with respect to their average, were combined with the appropriate value of Student's t-statistic to yield 99% marginal inference intervals, and only those parameters with estimates larger than such inference intervals were considered for further study.

Results and Discussion

Chromatographic analyses: Typical chromatograms obtained for the samples of *bagaceira* analyzed are presented in Figures 1 and 2. The results obtained for the concentration of the major volatile components in the distillate fractions are depicted in Tables 3 and 4.

As shown in Tables 3 and 4, fusel alcohols are quantitatively the largest group of flavor compounds in the samples analyzed, with a range of 395 - 2029 mg/L. This group is composed of aliphatic alcohols (1-propanol, 2-methyl-propanol, 2-methyl-butanol, and 3-methyl-butanol) and aromatic alcohols (especially 2-phenyl-ethanol), which can be generated from amino acids. Due to their high volatilities, fusel alcohols are preferentially concentrated in the first distillates, where they are responsible for a pungent smell and taste.

The group containing the highest number of different components that contribute to the flavor of *bagaceiras* (as happens with most spirits) is fatty acid esters, with five compounds. As volatile compounds which account for a pleasant smell, they are of great importance to the overall quality of spirits, although key aromas can rarely be associated with only a specific ester. Ethyl acetate, as well as ethyl hexanoate, ethyl octanoate, ethyl decanoate, and ethyl dodecanoate, are distilled early; in contrast, ethyl lactate is recovered in the condensate by the end of distillation. Ethyl acetate and ethyl lactate are quantitatively the most important esters present in *bagaceiras*, and their ranges are 176 - 9614 and 0 - 310 mg/L, respectively; 2-phenyl-ethyl-acetate is the less representative one, with the range 0-1 mg/L. Either due to their toxicity (*e.g.*, methanol) or to their poor organoleptic properties (*e.g.*, 2-butanol), the contents of alcohols should be combined with those of esters, fatty acids, and aldehydes in order to determine the time cuts between head products (*cabeças*), heart products (*coração*), and tail products (*caudas*). Components that can typically be characterized as head products include ethanal, ethyl acetate, 1-propanol, 2-methyl-propanol, 2-methyl-butanol, and 3-methyl-butanol; hexanol is typically a heart product; finally, 2-phenyl-ethanol, diethyl succinate, and ethyl lactate are typically characterized as tail products [9,10,14]. A convenient index of bacterial spoilage in marc distillates is high levels of such esters as ethyl acetate, ethyl lactate, and diethyl succinate, and such alcohols as 1-propanol, 1-butanol, and 2-butanol.

Methanol found in *bagaceiras* may, depending on the amount ingested, lead to blindness and even death. It is formed from pectins, the long-chain gum-like binding material originating from the walls of the berry cells in the crushed grapes, by enzymes which split the methoxyl group. When a (commercial) pectinase is deliberately added to the pomace, its pectin is broken down more rapidly than in the presence of native pectinases only, and consequently liberates galacturonic acid and methanol to the significant extents detected in distilled spirits [2,6,9]. Hence, the rate of such degradation is enhanced by the extent of maceration of the pomace and the total concentration of pectinase (as experimentally observed). The concentration of methanol in the final product, which ranges from 2694 to 6960 mg/L, also increases as the splitting between *cabeças* and *coração* is made earlier.

Statistical analyses: The empirical models fitted to the data generated for both grape varieties after elimination of effects that are not statistically significant on the 1% level are depicted in Table 5 for the intermediate distillate fraction (*i.e.*, the one actually sold as *bagaceira*); those obtained for the head and tail products are not shown for the sake of bookkeeping.

The statistical adequacy of using minimum least squares to estimate the various adjustable parameters in the proposed empirical models was assessed through diagnostic plots; since plots of the residuals *vs.* the percentiles of a normal distribution did not show deviations from linearity, and plots of residuals *vs.* the average of each set of two replicates did not exhibit funnel-shaped or other biased tendencies, it could be concluded that the errors were normally distributed and the variance was constant, thus ensuring validity for the reasoning utilized.

From the global analysis of the magnitude of the significant parameter estimates listed in Table 5, it is interesting to note that the most volatile alcohols (*viz.* propanol, 2-methyl-propanol, 2-methyl-butanol, and 3-methyl-butanol) are considerably influenced by the fermentation time variable with a clear negative effect; *i.e.*, alcohol synthesis is eventually followed by alcohol consumption as fermentation time elapses. The more important factor for 2-butanol appearance is the type of container; apparently, the higher degrees of aerobiosis and the possibilities for clustering of lactic acid bacteria within the wooden container promote bacterial contamination; it should as well be noted that lactic acid bacteria, mostly responsible for 2-butanol synthesis, reach their highest numbers after one to two weeks [13]. As expected in view of the reasoning detailed above, the most important positive effect on methanol formation is pectinase addition; conversely, tartaric acid addition has a negative effect on methanol forma-

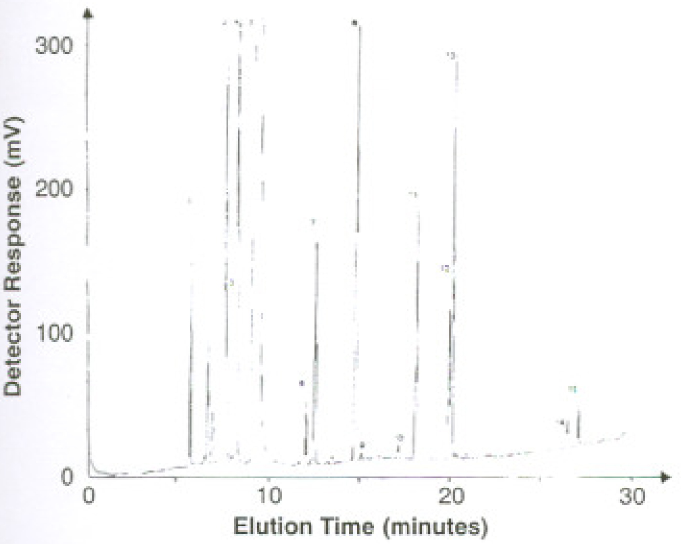

Fig. 1. Typical gas chromatogram obtained after direct injection of *coração* of a bagaceira of Loureiro. 1, ethanal; 2, ethyl acetate; 3, acetal; 4, methanol; 5, ethanol; 6, 2-butanol; 7, 1-propanol; 8, 2-methyl-1-propanol; 9, allylic alcohol; 10, 1-butanol; 11, 4-methyl-2-pentanol (internal standard);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

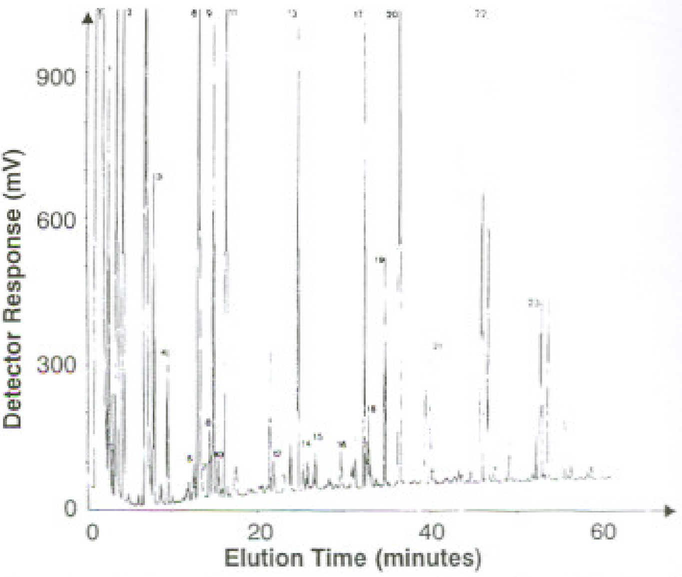

Fig. 2. Typical gas chromatogram obtained after direct injection of *coração* of a bagaceira of Loureiro. 1, ethyl butyrate; 2, isoamyl acetate; 3, ethyl hexanoate; 4, hexyl acetate; 5, ethyl lactate; 6, hexanol; 7, *trans*-3-hexenol; 8, *cis*-3-hexenol; 9, 3-octanol (internal standard); 10, *trans*-2-hexenol; 11, ethyl octanoate; 12, isobutyric acid; 13, ethyl decanoate; 14, isovaleric acid; 15, diethyl succinate; 16, 2-phenylethylacetate; 17, ethyl dodecanoate; 18, hexanoic acid; 19, 2-phenylethanol; 20, heptanoic acid (internal standard); 21, octanoic acid; 22, decanoic acid; 23, dodecanoic acid.

tion. Tartaric acid has also an influence on allylic alcohol (negative effect) and 2-phenyl-ethanol (positive effect). Alcohols not affected by any of the manipulated variables considered are 1-butanol, *trans*-3-hexenol, and *cis*-3-hexenol. With respect to carboxylic acids, it is important to note that for longer chain acids the effects of all manipulated variables studied, except fermentation time, are negative; fermentation time does not considerably affect acid formation except for propionic acid; isoamylic acid is positively influenced by enzyme addition and, to a lesser extent, by container type. In what concerns esters, the important factors depend on the particular ester in question: the type of container is more important for isoamyl acetate and hexyl acetate,

Table 3. Experimental data for concentration (mg/L) of major volatiles obtained from distillation of *Alvarinho* grape pomace, averaged over the three cuts.

Ref. #	Exper. run	1	2	3	4	5	6	7	8	Mean	1% statistically significant factors
1	Methanol	4839	4909	4594	4354	4707	4616	4435	4596	4631	—
2	Ethanal	333.6	302.8	374.0	474.3	395.6	179.0	313.2	333.5	338.3	—
3	Acetal	89.6	58.63	75.59	78.36	62.66	36.34	72.57	94.6	71.03	1, 3, 1×2, 2×3, 1×2×3
4	2-Butanol	3.391	3.080	0.0000	0.0000	1.733	8.630	0.0000	0.0000	2.104	1, 3, 1×2, 1×3, 2×3, 1×2×3
5	1-Propanol	233.0	216.2	236.9	215.1	219.2	212.8	223.3	231.7	223.5	1, 2, 3, 1×2, 1×3, 2×3,1×2×3
6	2-Methyl-propanol	363.8	324.0	381.9	347.9	333.5	335.6	332.8	355.8	346.9	1, 2, 3,1×2, 1×3, 2×3, 1×2×3
7	1-Butanol	9.160	9.026	11.04	11.37	10.84	8.220	11.46	10.63	10.22	1, 3,1×2, 2×3, 1×2×3
8	2-Methyl-butanol	184.3	171.6	195.0	177.8	166.2	165.8	173.9	186.1	177.6	—
9	3-Methyl-butanol	446.8	406.7	464.9	444.7	424.4	402.2	685.9	454.5	466.2	1, 2, 3, 1×2×3
	TOTAL (4 to 9)	1240	1131	1290	1197	1156	1133	1427	1239	1227	
10	Allylic alcohol	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	—
11	Hexanol	89.18	85.45	94.92	97.28	82.66	85.13	123.3	130.4	98.54	2, 2×3, 1×2×3
12	*Trans*-3-hexenol	0.1504	0.10269	0.01540	0.03763	0.2600	0.2306	0.2047	0.3755	0.1721	—
13	*Cis*-3-hexenol	0.5599	0.5327	0.7050	0.973	0.5069	0.8109	1.255	0.6022	0.7432	1, 3, 1×2, 1×3, 2×3, 1×2×3
14	*Trans*-3-hexenol	0.3136	0.1757	1.679	0.4120	0.2053	0.3024	1.372	3.206	0.9582	1×2
15	2-Phenyl-ethanol	6.684	6.539	5.018	6.641	4.411	5.511	7.477	4.979	5.908	1, 2, 3, 1×2
16	Propionic acid	6.213	6.318	21.36	17.58	23.62	22.59	10.22	15.55	15.43	1, 2, 3, 1×2, 2×3
17	Isobutyric acid	2.782	2.177	0.8066	1.207	1.831	2.277	2.905	0.8850	1.859	1, 2, 3, 1×2, 1×3, 2×3, 1×2×3
18	Isoamylic acid	1.383	0.5995	0.4724	0.5732	1.088	0.9030	1.115	1.217	0.919	1, 2, 3, 1×2, 1×3, 2×3, 1×2×3
19	Hexanoic acid	1.556	1.561	1.364	1.447	1.345	1.526	2.618	1.457	1.609	1, 3, 1×2, 1×3, 2×3, 1×2×3
20	Octanoic acid	1.310	1.640	1.658	1.708	1.181	1.854	2.223	1.554	1.641	3, 1×3
21	Decanoic acid	0.9477	1.394	3.515	1.817	0.9440	1.564	2.226	0.9272	1.667	1
22	Dodecanoic acid	0.1293	0.2372	0.961	0.3414	0.6118	0.7258	1.089	0.9510	0.6307	1, 2, 3, 1×2, 1×3, 2×3, 1×2×3
	TOTAL (19 to 22)	3.943	4.833	7.498	5.314	4.082	5.670	8.156	4.889	5.548	
23	Ethyl acetate	2093	1749	2542	1170	1530	927.7	778.7	541.0	1416	1,2,3,1×2,1×3,2×3,1×2×3
24	Isoamyl acetate	8.555	14.96	3.707	1.047	3.584	1.2233	0.9330	0.6489	4.333	1, 2, 3,1×2, 1×3, 2×3, 1×2×3
25	Hexyl acetate	2.362	1.859	1.306	0.3273	0.6867	0.5520	0.2105	0.4243	0.9660	2, 3, 1×2, 1×2×3
26	2-Phenyl-ethyl-acetate	0.01509	0.005363	0.01379	0.008797	0.02524	0.01749	0.0000	0.06703	0.01910	1, 2, 3, 1×2, 2×3, 2×3
	TOTAL (24 to 26)	10.93	16.83	5.027	1.383	4.296	1.793	1.144	1.140	5.318	
27	Ethyl butyrate	0.2784	0.2109	0.2304	0.04282	0.2540	0.05904	0.1098	0.08199	0.1584	—
28	Ethyl hexanoate	1.491	1.496	1.094	0.8828	0.7768	0.9485	0.8627	1.002	1.069	2
29	Ethyl octanoate	3.325	3.588	2.629	0.994	1.153	1.767	1.292	1.272	2.002	1, 2, 1×2, 1×3, 2×3, 1×2×3
30	Ethyl decanoate	2.128	2.208	4.499	0.4941	0.6805	1.482	1.688	1.335	1.814	1, 2, 3, 1×2, 1×3, 2×3, 1×2×3
31	Ethyl dodecanoate	0.3739	0.3443	1.819	0.3857	0.2897	0.7992	0.7024	0.7522	0.6833	
	TOTAL (27 to 31)	7.596	7.847	10.27	2.799	3.154	5.055	4.654	4.444	5.728	—
32	Diethyl succinate	2.408	2.387	0.6625	1.094	2.107	2.545	1.701	2.127	1.879	1, 2, 3, 1×2, 1×3, 2×3
33	Ethyl lactate	235.7	264.5	156.1	188.2	240.3	251.8	196.5	193.5	215.8	2, 3, 1×2, 1×3, 2×3, 1×2×3

Note: 1 - normalized level of addition of tartaric acid, defined as (T-40)40; 2 - normalized time of fermentation, defined as (t-31.5)/10.5; 3 - normalized level of pectinase addition, defined as (E-1); **1× 2; 1× 3 ; 2× 3, 1× 2× 3** - interaction effects between the manipulated variables.

with plastic better then wood; tartaric acid addition has a positive effect on 2-phenyl-ethyl-acetate and ethyl butyrate, but a negative effect on ethyl lactate, hexanoate, and decanoate; fermentation time has a positive effect on ethyl octanoate; and pectinase addition has a positive effect on ethyl dodecanoate and diethyl succinate. Ethyl acetate, ethanal, and acetal contents are known to positively correlate with bacterial charge [1], and so, as observed in this research program, it is anticipated that the container has a significant effect. It would in principle be expected that formation of alcohols should be influenced in a similar fashion by the same factors because all of them (except methanol and 2-butanol) have a common origin (*i.e.*,

Table 4. Experimental data for concentration (mg/L) of major volatiles obtained from distillation of *Loureiro* grape pomace, averaged over the three cuts.

Ref. #	Exper. run	1	2	3	4	5	6	7	8	9	10	Mean	1% statistically significant factors
1	Methanol	4406	4011	4590	3838	5215	4831	4635	4527	4825	5527	4641	1
2	Ethanal	270.1	227.7	322.0	331.3	212.9	225.7	35635	229.9	299.5	671.9	314.7	—
3	Acetal	265.3	232.1	279.4	241.7	181.0	254.7	198.1	226.7	400.8	1042	332.2	—
4	2-Butanol	3.080	1.517	7.177	2.427	7.037	7.780	3.040	3.013	9.500	74.45	11.90	2
5	1-Propanol	196.3	201.5	199.0	202.0	197.6	210.2	213.5	187.4	189.9	203.3	200.1	—
6	2-Methyl-propanol	405.7	399.5	381.7	400.9	407.1	389.6	416.9	344.3	360.3	321.5	382.8	2,4
7	1-Butanol	1.3767	5.200	3.663	3.397	2.173	5.357	0.000	0.000	0.000	3.990	2.516	2,4 ,1x2, 2x3
8	2-Methyl-butanol	239.2	229.0	216.6	242.0	255.3	232.5	250.9	207.3	189.1	233.0	226.5	—
9	3-Methyl-butanol	402.1	400.6	375.8	423.6	384.3	406.4	450.6	297.1	335.3	432.7	390.8	1x2
	TOTAL (4 to 9)	1248	1237	1184	1274	1223	1252	1335	1039	1084	1269	1215	
10	Allylic alcohol	2.123	6.393	3.733	0.000	10.400	0.0000	12.22	13.05	0.0000	8.337	5.626	—
11	Hexanol	48.87	51.84	51.72	42.03	51.85	55.04	61.32	49.21	43.85	58.21	51.39	—
12	*Trans*-3-hexenol	0.4110	0.3010	0.1825	0.2023	0.2037	0.1129	0.2635	0.02289	0.1470	0.1298	0.1977	2, 4,1x3
13	*Cis*-3-hexenol	0.3331	0.1302	0.1003	0.1488	0.1253	5.687	0.2480	0.5363	7.428	4.005	1.874	1, 3,1x2, 2x3,
14	*Trans*-3-hexenol	0.08768	2.413	0.000	0.04583	2.499	2.895	0.00776	0.01989	2.066	2.134	1.217	1, 2, 1x2
15	2-Phenyl-ethanol	5.092	5.562	5.547	4.477	5.120	4.910	5.062	4.469	5.010	4.459	4.971	—
16	Propionic acid	20.58	16.89	13.54	8.65	10.92	3.883	1.802	1.208	1.546	2.647	8.167	3, 4, 1x2
17	Isobutyric acid	1.283	2.090	3.310	0.6744	0.9380	0.9330	0.8196	0.2821	1.020	0.7564	1.193	—
18	Isoamylic acid	1.314	1.426	0.7960	0.8920	1.054	0.3512	0.909	0.1230	0.5717	1.360	0.880	1, 2, 1x2
19	Hexanoic acid	1.221	1.177	1.067	0.7939	1.104	0.6686	0.6427	1.095	0.5819	1.080	0.9430	2
20	Octanoic acid	1.092	1.106	0.5821	0.6964	0.6350	0.7510	0.4367	1.169	0.3667	0.5127	0.7348	3, 1x3
21	Decanoic acid	0.6689	0.6609	0.3901	0.3626	1.013	0.4661	0.7855	2.218	0.5811	0.8760	0.7931	2x3
22	Dodecanoic acid	0.4552	0.5153	0.4147	0.5079	0.4663	0.3143	0.1369	0.6645	0.1493	0.2201	0.3844	—
	TOTAL (19 to 22)	3.437	3.459	2.454	2.361	3.218	2.200	2.002	5.057	1.679	2.689	2.855	
23	Ethyl acetate	2557	1680	1629	3601	1639	2595	2466	1188	2050	2825	2223	2, 1x2
24	Isoamyl acetate	2.509	1.840	1.046	2.579	1.108	1.817	2.945	1.104	1.078	1.971	1.800	1
25	Hexyl acetate	0.6323	0.2236	0.4250	0.5904	0.2271	0.5185	0.7653	0.07520	0.1221	0.8215	0.4400	1, 3
26	2-Phenyl-ethyl-acetate	0.2929	0.3168	0.3880	0.2244	0.3236	0.1341	0.1499	0.05951	0.2206	0.2230	0.2333	4, 1x2, 2x3
	TOTAL (24 to 26)	3.435	2.380	1.859	3.394	1.659	2.470	3.861	1.238	1.421	3.016	2.473	
27	Ethyl butyrate	0.1094	0.1578	0.3105	0.1468	0.1653	0.0740	0.0619	0.0164	0.0691	0.05278	0.1164	1, 2x3
28	Ethyl hexanoate	0.8341	0.6505	0.6954	0.6338	0.6299	0.7069	1.143	0.7620	0.8144	1.144	0.8015	—
29	Ethyl octanoate	0.7115	0.5202	0.5038	0.3772	0.4277	0.3615	0.5148	0.4379	0.2650	0.5574	0.4677	4
30	Ethyl decanoate	0.7078	0.5017	0.6105	0.5787	0.6276	0.7464	0.6625	1.638	0.3995	0.6894	0.7162	1, 2, 4, 1x2, 1x3
31	Ethyl dodecanoate	0.5550	0.6614	0.6292	0.5984	0.6247	0.6980	0.4587	0.7624	0.2525	0.6843	0.5925	1
	TOTAL (27 to 31)	2.918	2.492	2.749	2.335	2.475	2.587	2.841	3.616	1.800	3.128	2.694	
32	Diethyl succinate	3.725	2.524	3.371	1.794	2.961	2.167	2.512	10.29	2.957	2.369	2.541	2, 3,4, 1x3,2x3
33	Ethyl lactate	45.16	0.000	20.07	23.32	75.64	38.53	48.64	81.70	34.77	103.7	47.16	1, 2, 3, 4, 1x2,1x3,2x3

Note: **1** - normalized level of addition of tartaric acid, defined as (T-40)40; **2** - normalized time of fermentation, defined as (t-28)/7; **3** - normalized level of pectinase addition, defined as (E-1); **1× 2**; **1× 3** ; **2× 3**, **1× 2× 3** - interaction effects between the manipulated variables.

Table 5. Empirical models (with explicit indication of means of each significant effect) for estimation of concentration, Cc (in mg/L), of major volatiles in heart distillates obtained from *Alvarinho* and *Loureiro* grape pomaces.

Component	Alvarinho	Loureiro
Methanol	$Cc = 4883.94$	$Cc = 4737.49 - 396.49\ x_1$
1-Butanol	$Cc = 11.23$	$Cc = 2.99$
Propanol	$Cc = 231.89 - 10.77\ x_1 - 11.02\ x_3 - 12.42\ x_1x_2 - 9.94\ x_2x_3 + 11.72\ x_1x_2x_3$	$Cc = 207.13$
2-Methyl-Propanol	$Cc = 361.06 - 6.79\ x_1 - 20.27\ x_3 - 35.67\ x_1x_2 + 13.88\ x_1x_3 - 16.32\ x_2x_3 + 25.76\ x_1x_2x_3$	$Cc = 368.81 + 21.81\ x_2$
2-Butanol	$Cc = 1.84 - 1.84\ x_1 + 1.84\ x_2 - 1.84\ x_3 - 1.84\ x_1x_2 + 1.84\ x_1x_3 - 1.84\ x_2x_3 + 1.84\ x_1x_2x_3$	$Cc = 4.47$
2-Methyl-butanol	$Cc = 182.54 - 3.61\ x_1 - 2.26\ x_2 - 11.28\ x_3 - 14.59\ x_1x_2 + 5.87\ x_1x_3 - 8.28\ x_2x_3 + 12.25\ x_1x_2x_3$	$Cc = 243.62 + 14.8\ x_2 + 14.39\ x_4$
3-Methyl-butanol	$Cc = 444.85 - 14.50\ x_1 - 29.65\ x_3 - 37.55\ x_1x_2 - 13.22\ x_2x_3 + 30.25\ x_1x_2x_3$	$Cc = 427.80 + 41.15\ x_2 + 41.22\ x_4 - 34.56\ x_1x_2 - 34.76\ x_2x_3$
Allylic alcohol	$Cc = 0.00$	$Cc = 8.63$
Hexanol	$Cc = 100.38 - 11.78\ x_1 - 11.94\ x_2 - 8.59\ x3 + 7.91\ x_1x_2x_3$	$Cc = 64.74 + 3.94\ x_1x_2$
Trans-3-hexenol	$Cc = 0.54$	$Cc = 0.00$
Trans-2-hexenol	$Cc = 0.90 - 0.67\ x_2 + 0.71\ x_2x_3 - 0.61\ x_1x_2x_3$	$Cc = 0.24$
Cis-3-hexenol	$Cc = 0.54$	$Cc = 0.03 + 0.03\ x_2 + 0.02\ x_4 + 0.03\ x_1x_3$
2-Phenyl-ethanol	$Cc = 5.02618 + 0.2789\ x_1 + 0.618\ x_3 + 1.3266\ x_1x_2 - 0.6625\ x_1x_3 - 0.6035\ x_2x_3 + 0.3629\ x_1x_2x_3$	$Cc = 3.63 + 0.16\ x_1 + 0.15\ x_3 + 0.28\ x_1x_2 - 0.14\ x_2x_3$
Propionic acid	$Cc = 17.85 - 10.96\ x_1x_2$	$Cc = 2.73 + 2.42\ x_1 + 3.41\ x_2 + 2.42\ x_1x_2$
Isobutyric acid	$Cc = 1.23 + 0.49\ x_1 + 0.48\ x_2 + 0.23\ x_3 + 0.70\ x_1x_2$	$Cc = 0.23$
Isoamylic acid	$Cc = 0.49 + 0.13\ x_1 + 0.21\ x_2 + 0.21\ x_3 + 0.17\ x_1x_2 + 0.09\ x_2x_3$	$Cc = 0.21 + 0.10\ x_3 + 0.08\ x_4 + 0.18\ x_1x_2$
Hexanoic acid	$Cc = 1.24 - 0.06\ x_1 - 0.17\ x_2 + 0.29\ x_3 + 0.44\ x_1x_2 - 0.29\ x_1x_3 - 0.26\ x_2x_3 + 0.16\ x_1x_2x_3$	$Cc = 0.36$
Octanoic acid	$Cc = 1.37 - 0.05\ x_1 - 0.04\ x_2 + 0.06\ x_3 + 0.22\ x_1x_2 - 0.23\ x_1x_3 - 0.26\ x_2x_3 + 0.19\ x_1x_2x_3$	$Cc = 0.35 + 0.13\ x_1 + 0.13\ x_2 + 0.19\ x_1x_2$
Decanoic acid	$Cc = 1.00 + 0.13\ x_1 - 0.15\ x_3 + 0.13\ x_1x_2 - 0.15\ x_1x_3 - 0.15\ x_2x_3 + 0.08\ x_1x_2x_3$	$Cc = 0.35 + 0.15\ x_2$
Dodecanoic acid	$Cc = 0.16 - 0.02\ x_3 - 0.03\ x_1x_3$	$Cc = 0.11 - 0.03\ x_3 + 0.03\ x_1x_3$
Ethyl butyrate	$Cc = 0.13 + 0.06\ x_1$	$Cc = 0.06 - 0.03\ x_2x_3$
Ethyl hexanoate	$Cc = 0.86 + 0.12\ x_1 + 0.34\ x_2 - 0.07\ x_3 + 0.11\ x_1x_2 + 0.10\ x_1x_3 - 0.17\ x_2x_3 + 0.18\ x_1x_2x_3$	$Cc = 0.52$
Ethyl octanoate	$Cc = 1.70 + 0.58\ x_1 + 1.09\ x_2 - 0.17\ x_3 + 0.61\ x_1x_2 + 0.16\ x_1x_3 - 0.34\ x_2x_3 + 0.30\ x_1x_2x_3$	$Cc = 0.43 + 0.11\ x_2 + 0.1\ x_1x_2$
Ethyl decanoate	$Cc = 0.98 + 0.35\ x_1 + 0.81\ x_2 - 0.25\ x_3 + 0.33\ x_1x_2 + 0.22\ x_1x_3 - 0.28\ x_2x_3 + 0.31\ x_1x_2x_3$	$Cc = 0.38 - 0.28\ x_1$
Ethyl dodecanoate	$Cc = 0.23 + 0.07\ x_2 - 0.08\ x_3 - 0.06\ x_1x_2 + 0.09\ x_1x_2x_3$	$Cc = 0.30 - 0.17\ x_1 - 0.11\ x_3$
Ethyl acetate	$Cc = 1246.65 + 422.66\ x_1 + 146.81\ x_2 + 170.81\ x_3 - 291.97\ x_1x_2 + 142.52\ x_1x_3 - 164.70 x_2x_3$	$Cc = 1257.88 + 338.04\ x_4 - 151.56\ x_1x_2 - 218.53\ x_2x_3$
Ethyl lactate	$Cc = 233.02$	$Cc = 47.08 - 21.08\ x_1 + 12.89\ x_2x_3$
Diethyl succinate	$Cc = 1.43 + 0.59\ x_2$	$Cc = 1.25$
Hexyl acetate	$Cc = 0.72 + 0.41\ x_1 + 0.64\ x_2 + 0.34\ x_1x_2 + 0.21\ x_1x_3 - 0.09\ x_2x_3 + 0.14\ x_1x_2x_3$	$Cc = 0.13 + 0.10\ x_4$
Isoamyl acetate	$Cc = 2.86 + 1.42\ x_1 + 1.85\ x_2 + 1.09\ x_3 + 0.91\ x_1x_2 + 0.71\ x_1x_3 + 0.43\ x_2x_3 + 0.39\ x_1x_2x_3$	$Cc = 1.12 + 0.26\ x_1 + 0.22\ x_2 + 0.24\ x_4 + 0.45\ x_1x_2 - 0.26\ x_1x_3$
2-Phenyl-ethyl-acetate	$Cc = 0.01$	$Cc = 0.08 - 0.06\ x_1$
Ethanal	$Cc = 367.11 + 48.53\ x_1 - 33.58\ x_2 + 27.31\ x_3 - 44.06\ x_1x_2 - 41.82\ x_1x_3 + 44.32\ x_2x_3$	$Cc = 246.68 - 35.06\ x_2 + 24.29\ x_3 + 42.37\ x_4 -$ $-25.19\ x_1x_3 - 15.91\ x_2x_3$
Acetal	$Cc = 51.26 - 3.29\ x_2 - 7.43\ x_3 - 11.91\ x_1x_2 - 2.72\ x_1x_3 + 3.59\ x_2x_3 + 10.21\ x_1x_2x_3$	$Cc = 84.24 + 25.86\ x_1 + 11.9\ x_2 - 25.86\ x_3$ $+ 36.33\ x_4 - 11.9\ x_1x_2 + 25.86\ x_1x_3 - 11.9\ x_2x_3$

yeasts fermentation); however, this was not observed.

Comparing the results pertaining to the methanol contents of the two varieties, it can be concluded that there is little difference between the various distillate fractions for *Loureiro*, whereas a clear separation between head, heart, and tail products exists for *Alvarinho*. Although the alternative sources of heating, the heterogeneity of the grape pomace, and the different proportions of water added might account partially for the disparate results obtained for the two varieties, most variability is likely due to the grape variety itself.

Conclusions

With respect to volatile compounds that pose health or organoleptic hazards, *viz.* methanol and 2-butanol, the effect of pectinase addition is statistically the most significant for the methanol content, whereas the effect of fermentation time is statistically the most significant for the 2-butanol content. Addition of pectinase plays also a statistically significant role in terms of propanol, 2-methyl-propanol, 2-methyl-butanol, 3-methyl-butanol, hexanoic acid, octanoic acid, acetal, ethyl decanoate, and ethyl dodecanoate contents. The effect of tartaric acid addition appears to be the most relevant for *trans*-3-hexenol, *trans*-2-hexenol, isoamylic acid, isoamyl acetate, hexyl acetate, ethyl acetate, ethyl butyrate, and ethyl octanoate. The contents of the remaining components are mostly affected by fermentation time.

This work is useful because it allows, to a certain extent, prediction of how the concentration of every major volatile responds to each manipulated processing factor in the distillate, a preliminary step to rationalization of the process of manufacture of *bagaceiras*.

Although practical experience has indicated that the chemical composition, and thus the typical flavor, of a spirit are tightly associated with the conditions prevailing during winemaking, storage, and distillation, the similarities of *bagaceiras* with Orujo, Marc, Tsipouro, and Grappa (in terms of raw material and production techniques) allow the results reported here for *bagaceiras* to be (somehow) extrapolated to such spirits.

Literature Cited

1. Amerine, M. A., and C. S. Ough. Methods for Analysis of Musts and Wines. pp 74-152. Wiley, New York (1980).

2. Belchior, A. P. Factores que condicionam os teores de metanol dos bagaços. Enologia 2:1-9 (1980).

3. Bertrand, A. Recherches sur l´analyse des vins par chromatographie en phase gazeuse. Thèse Docteur en Sciences. Université de Bordeaux II, France (1975).

4. Bertrand, A. Role of the continous distillation process on the quality of Armagnac. *In:* Distilled Beverage Flavour. Recent Developments (J. R. Piggott and Paterson, Eds.). pp 97-115. Ellis Morwod, Edinburgh (1988).

5. Box, G. E. P., W. G. Hunter, and J. S. Hunter. Statistics for Experimenters. Wiley, New York (1978).

6. Lee, C. Y., W. B. Robinson, J. P. Van Buren, T. E. Acree, and G. S. Stoewsand. Methanol in wines in relation to processing and variety. Am. J. Enol. Vitic. 36:184-187 (1975).

7. Monteiro, F. F., and N. Pereira. Pectinas e actividade pectinolitica. Perspectiva enológica. Bol. Biotecnol. 54: 27-35 (1996).

8. Mourão, L., and I. Marrana. Legislação Vitivinícola Comunitária, Vol. III. Instituto do Vinho do Porto, Porto, Portugal (1990).

9. Orriols-Fernandez, I., B. Rodriguez, and J. F. Perez-Fernandez. L´eau-de-vie de marc ("aguardente") d´albarino: Influence de trois systèmes de distillation sur les teneurs en composés volatiles. *In:* Les Eaux-de-vie Traditionelles d´Origine Viticole. A. Bertrand (Ed.). pp 43-49. Lavoisier, Paris (1991).

10. Orriols-Fernandez, I. Tecnologia de la destilacion en los aguardientes de orujo. Proc. Congreso Internacional de la Viticultura Atlántica. pp 291-324. (Spain) (1994).

11. Rankine, B. Making good wine. Sun, Australia (1989).

12. Silva, M. L., F. X. Malcata, and G. Revel. Volatile contents of grape marcs in Portugal. J. Food Comp. Anal. 9:72-80 (1996).

13. Silva, M. L., F. X. Malcata, and T. Hogg. A. How do processing conditions affect the microflora of the sweet pomace of white grapes from Vinho Verde? Food Sci. Technol. Int. 1:129-136 (1996).

14. Soufleros, E. La production artisanale du "Tsipouro" a Naoussa (Grèce). *In:* Les Eaux-de-vie Traditionelles d´Origine Viticole. A. Bertrand (Ed.). pp 19-27. Lavoisier, Paris. (1991).

15. Usseglio, T. Le caractteristiche della grappa. Vini d'Italia. 453-462 (1971).

16. Varajão, G. A. Les eaux de vie de marc du Portugal. *In:* Les Eaux-de-vie Traditionelles d'Origine Viticole. A. Bertrand (Ed.). pp 38-41. Lavoisier, Paris (1991).

17. Versini, G. Risultati de una sperimentazione per l'inibizione della formazione del 2 butanolo nella conservazione della vinaccia. pp 49-54. Proc. Asti Convegno Nazionale Grappa, C. C. I. A. A., Asti (1981).

18. Versini, G., and L. Odello. La grappa: Considérations sur la distillation traditionnelle en Italie. *In:* Les Eaux-de-vie Traditionelles d'Origine Viticole A. Bertrand (Ed.). pp 32-37. Lavoisier, Paris (1991).

19. Versini, G., A. Monetti, A. Dalla Serra, and S. Inama. Analytical and statistical characterization of "grappa" from different Italian regions. *In:* Les Eaux-de-vie Traditionelles d´Origine Viticole A. Bertrand (Ed.). pp 137-150. Lavoisier, Paris (1991).