Effects of Grape Destemming on the Polyphenolic and Volatile Content of Fino Sherry Wine during Alcoholic Fermentation

P. Benítez, R. Castro,* R. Natera and C. García-Barroso

Analytical Chemistry Department, Faculty of Sciences, University of Cádiz, Polígono Río San Pedro s/n, P.O. Box 40, 11510 Puerto Real, Cádiz, Spain

This study has been conducted to explore the influence of different degrees of destemming on the polyphenolic and volatile content of wines from the *Palomino fino* grape variety. Wines obtained from destemmed grape did not present reduced polyphenolic content. No relationship was observed between degrees of destemming and changes in the polyphenolic content. Destemming prior to pressing did not modify the production of volatile compounds during fermentation and wines with similar organoleptic characteristics to those without destemming were obtained.

Key Words: destemming, fermentation, polyphenolic compounds, volatile compounds, fino sherry wine

INTRODUCTION

The main function of destemming is to separate the grape berries from their stalks and other associated vegetable matter. For red wines, it is well-known that the influence of destemming on the composition and quality of wines is very important. Red wines made from non-destemmed grape generally contain higher levels of phenolic compounds (Sun et al., 2001) but scarce information is found about white wines.

The term 'phenolic compounds' includes phenolic acids, flavanols, flavonols, flavanonols, proanthocyanidins and anthocyanins, which are responsible for many properties such as colour, browning, bitterness and astringency, in fruit, juices and fermented beverages. All of these compounds can be divided into two groups, flavonoids and nonflavonoids. Polymers of some of these phenols are termed tannins. These can be divided between hydrolysable and condensed groups. The first group include gallotannins and ellagitannins that release gallic acid and ellagic acid, respectively, after acid hydrolysis. They also contain a glucose molecule. The condensed tannins, also known as proanthocyanidins cannot easily be decomposed by hydrolysis. In this group procyanidins can also be found.

**To whom correspondence should be sent (e-mail: remedios.castro@uca.es).*
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All these phenolic compounds are more or less reactive and, depending on their relative amounts in the grape, lead to various products formed by enzymatic or chemical reactions during food processing. The new constituents formed may have different properties from those of their precursors.

White wines exhibit high colour instability as a result of the oxidation process (Singleton, 1987). Flavanol monomers and oligomers play an important role in oxidation reactions (Macheix et al., 1991) that characterise the process of browning in white wine.

The most important factors affecting the content of these compounds in wine are their concentrations in the grape, the winemaking technology, and their transformation during the wine ageing process (Macheix et al., 1990). Hence, the winemaking technology plays an important role in enology since it influences directly on the sensory characteristics of the wine obtained (Christaki, 2002).

Grape stems contain significant amounts of polyphenolic compounds, especially phenolic acids, flavonols and flavanonols. Souquet et al. (2000) found that condensed tannin content of the stem was intermediate between that of the seed and the skin and that this variable could not be used to differentiate between red and white grape varieties. Revilla et al. (1997) observed that the content of catechins and procyanidins in red wine was affected by the destemming of grape clusters.

Generally all wines are made in a common process, with variations depending on the type to be produced.

Removal of stems before crushing has several advantages. Notably, it minimises the excessive uptake of phenols and lipids from vine parts. Stem phenols

generally produce more astringent and bitter tastes than phenols released by seed and skin.

Stems are often left with the must throughout fermentation, especially in the production of red wines. The higher content of polymers of both flavonoid and nonflavonoid compounds derived from a prolonged contact with the stems give red wines with improved colour density by stabilising the anthocyanin content (Castino, 1981). However, high flavonol content can also produce bitterness in white wines. The presence of stems made pressing easier presumably by creating drainage channels along which the juice could escape.

In white vinification, the destemming is not frequently practised because of the role of the stems in the drainage of the must after press. However, modern improvements in press design has made stem retention unnecessary, so in some cases, the destemming is now carefully carried out with the objective of avoiding the extraction of certain components which could provoke the development of undesirable sensorial characteristics. Besides, mechanical harvest that is actually imposing to manual harvest implies the elimination of stems.

Furthermore, the phenolic content of must may have various effects on the course of fermentation, thus determining the volatile content of the wine. Maceration with stems may increase the fermentation rate (Bréchot et al., 1971). Cantarelli (1989) found that the procyanidins can be slightly inhibitory of fermentation. In addition, phenols also might be modified by yeast action and produce certain volatile compounds.

Fermentation compounds principally higher alcohols and esters whose concentrations vary over a wide range, play an important role in white wine aroma (Rapp and Mandery, 1986; Schreier, 1979). They are by-products of yeast fermentation and their amounts in wine depend on many factors, such as yeast strain, grape must and fermentation conditions (Mallouchos et al., 2002).

According to their origin, two groups of wine alcohols can be found, those that are synthesised from a keto acid resulting from the oxidative deamination of an amino acid, or those involved as an intermediate in its biosynthesis, and those that are not produced directly from an amino acid, but from a keto acid that takes part as an intermediate in cell glucidic metabolism. However, Fraile et al. (2000) found that the formation of these alcohols occurred late in the fermentation, after the majority of amino acids had been consumed. This was explained on the basis of the production of alcohols during the biosynthesis of amino acids from the excess of their corresponding keto acids.

Aleixandre et al. (1998) observed that different contents in higher alcohols and esters were obtained in wines made from destemmed grape. In the case of fino sherry wine, production of the base wine generally follows standard white vinification procedures. Pressing almost immediately follows crushing to minimise tannin extraction. Tannins give a roughness inconsistent with accepted fino sherry regulations (Casas, 1985).

The objective of this study was to determine the influence of the destemming on the polyphenolic and volatile content of fino sherry.

MATERIALS AND METHODS

Samples and Vinification

Palomino fino variety grapes from the Jerez-Xérès-Sherry region (Southern Spain) were subjected to different degrees of destemming (0%, 25%, 50% and 75%). These proportions were obtained by blending grapes and destemming in appropriate proportions. A horizontal destemmer-crusher was employed.

Musts obtained by pressing at less than 1.5 kg/cm^2 were used. Before fermentation, musts were treated with tartaric acid (pH 3.5) and potassium metabisulphite (100 mg/L SO_2 equivalent). Then, the four musts were vinified under the same conditions (25 °C, inoculation with *Saccharomyces cerevisiae*). Fermentation curves were virtually identical for the four fermentations showing a lag time of approximately 24 h. The exponential phase was approximately of the same duration for all musts (approximately 8 days).

All of these musts were sampled periodically (0, 2, 4, 6 and 9 days) in order to determine their volatile and polyphenolic content.

Determination of Polyphenolic Compounds

Phenolic Acids

Eighty μL of each wine sample after filtration (0.45 μm pore size) was analysed by HPLC (Waters Cromatografía, S.A., Barcelona, Spain). The elution phases used were: solvent A (95% water, 5% methanol) and solvent B (95% methanol, 5% water) at pH 2.5 (extra pure sulphuric acid). The elution gradient was: from 100% to 85% solvent A in 5 min; from 85% to 50% solvent A in 40 min; and isocratic elution for 35 min. The analyses were carried out using a C_{18} column (Lichrospher 100 RP-18, 250 mm × 3 mm, 5 μm particle size) at a flow rate of 0.5 mL/min and detection at 280 and 320 nm. Analyses were carried out in triplicate.

Phenolic acids were identified by comparison with a library of DAD spectra and retention times of standards. Commercial standards of several polyphenols (gallic acid, caffeic acid and ferulic acid) were purchased from Fluka (Buchs, Switzerland). Other phenolic acid standards (syringic acid and *p*-coumaric acid) were supplied by Eastman Kodak (Rochester, NY). Caftaric and coutaric acids were isolated by the method described by Singleton et al. (1978). Each com-

pound was quantified by comparison with a calibration curve (absorbances at 320 nm for caftaric acid, *cis* and *trans* p-coutaric acids, fertaric acid, GRP, caffeic acid, *trans p*-coumaric acid and ferulic acid; absorbances at 280 nm for the other phenolic acids) obtained with the corresponding standard. GRP (2-S glutathionyl caftaric acid) and fertaric acid were quantified as caftaric acid and ferulic acid, respectively.

The precision was calculated using five analyses of a sample of wine. Coefficients of variation between 1.1 and 2.4% were obtained.

Flavan-3-ols

Extraction

A volume of 50 mL of each sample was concentrated under vacuum at 40 °C in order to eliminate the alcohol content. Then, each sample was submitted, in duplicate, to SPE, under the conditions detailed in Table 1. This process consisted of two stages, a prior stage of cleaning and preconcentration, and a fractionation stage. For the first stage, a volume of 10 mL of concentrated sample, after dilution to a final volume of 20 mL with a saturated NaCl solution at pH 2, was passed through a C18 cartridge (1 g, DSC-18, Supelco, Barcelona, Spain). The polyphenolic extract was eluted with methanol:phosphate buffer (1:1) at pH 6.5. This extract was passed through a SAX cartridge (500 mg, Bond Elux, Scharlau, Barcelona, Spain) after adjusting its pH value to 6.5. The cartridge was eluted with 1 mL of phosphate buffer (pH 6.5) and 2 mL of Milli-Q water. This volume, in addition to the volume obtained as a result of the sample runthrough prior to the elution, was used for the determination of the flavan-3-ols.

HPLC Analysis

Catechin, epicatechin and procyanidins B1 and B2 were quantified in this fraction. Eighty μL of each extract was analysed, in duplicate, by HPLC (Waters Cromatografía, S.A., Barcelona, Spain). The elution phases used were: solvent A (95% water, 5% methanol) and solvent B (95% methanol, 5% water) at pH 2.5 (extra pure sulphuric acid). The elution gradient was: from 100% to 75% solvent A in 5 min; and from 75% to 50% solvent A in 40 min. The analyses were carried out using a C_{18} column (Lichrospher 100 RP-18, 250 mm × 3 mm, 5 μm particle size) at a flow rate of 0.5 mL/min. Two detectors were used, a DAD (absorbance at 280 nm) and a fluorescence detector (excitation at 276 m and emission at 316 nm).

The flavanols present were identified by comparison with a library of DAD spectra and retention times of standards. Commercial standards were purchased from Extrasynthese (Genay, France). Each compound was quantified by comparison with a calibration curve (fluorescence signal) obtained with the corresponding standard.

The precision of this method was calculated using five extractions of a sample of wine. Coefficients of variation between 2.5 and 4.3% were obtained. The technique of standard additions was used in order to check the accuracy of this analytical method. A representative sample of wine was taken as matrix and known quantities of a global standard solution containing all the analytes were added at five levels and in duplicate. Recoveries close to 100% were obtained for all the flavanols.

Table 1. Solid-phase extraction for the determination of flavan-3-ols.

Adsorbent	Operation	Solvent
	Solvation	10 mL methanol
	Conditioning	10 mL saturated NaCl at pH 2
Cleaning and preconcentration stage. C18 (1 mg, DCS-18, Supelco)	Sample	10 mL wine with 10 mL saturated NaCl at pH 2
	Washing	1st: 2 mL saturated NaCl at pH 2 2nd: 2 mL 0.01 M HCl
	Elution of polyhenolic extract	2 mL Methanol/buffered phosphate solution at pH 6.5 (1/1)
Fractionation stage. SAX (500 mg, Bond Elut, Scharlau)	Conditioning	10 mL Milli-Q water
	Sample	1st: Polyphenolic extract 2nd: 1 mL buffered phosphate solution at pH 6.5
	Washing	2 mL Milli-Q water

Determination of Volatiles Content

SPME

SPME methodology was previously optimised in our laboratory (Castro et al., 2004). Briefly, for each SPME analysis, a volume of 25 mL of sample was pipetted and placed into a 50-mL glass vial with 3.0 g of NaCl. Each sample was spiked with 75 μL of a solution of 4-methyl-2-pentanol (2.516 g/L in Milli-Q water containing 15% v/v of ethanol) and equilibrated for 15 min at sampling temperature (40 °C). After this, the SPME fibre (CAR/PDMS, 85 μm) was inserted into the headspace. During the sampling time (45 min) the sample was stirred at constant speed. Each sample was analysed in triplicate.

Gas Chromatography

The samples were analysed using a GC 8000 chromatograph with a FID detector (Fisons Instruments, Milan, Italy). The injection was made in the splitless mode for 2 min. For the desorption of the analytes inside the GC injection port, the temperature was 280 °C.

The GC was equipped with a DB-Wax capillary column (J&W Scientific, Folsom, CA, USA), 60 m × 0.25 mm I.D., with a 0.25 μm coating. The carrier gas was helium at a flow rate of 1.1 mL/min. The detector temperature was 250 °C. The GC oven was programmed as follows: held at 35 °C for 10 min, then ramped at 5 °C/min to 100 °C. Then it was raised to 210 °C at 3 °C/min and held for 40 min.

The compounds were identified by mass spectrometric analysis. In these analyses, the same GC coupled to a MD 800 mass detector (Fisons Instruments, Milan, Italy) was used. The mass detector operated in EI+ mode at 70 eV in a range of 30 to 450 amu. GC analytical conditions were the same as described above.

The signal was recorded and processed with Masslab software supplied with the Wiley 6.0 MS library. Peak identification was carried out by analogy of mass spectra and confirmed by retention indices of standards. All standards used in this study were supplied by Sigma-Aldrich (St Louis, MO, USA). Each compound was quantified by comparison with a calibration curve, obtained using the relative peak area in relation to that of 4-methyl-2-pentanol, the internal standard.

Statistical Analysis

Analysis of variance (ANOVA) and principal component analysis (PCA) were performed using the Statgraphics Statistical Computer Package Statgraphics Plus 5.1 for Windows XP.

RESULTS AND DISCUSSION

Polyphenolic Compounds

The main polyphenolic compounds found for the four samples of musts 2 days after the inoculation and at the end of the alcoholic fermentation were caftaric acid, GRP (2-S glutathionyl caftaric acid), *trans p*-coutaric acid, procyanidin B2 and caffeic acid (Table 2).

Hydroxycinnamic esters were present in high concentrations, caftaric acid was the main polyphenol and fertaric acid the minor one.

Only gallic acid and syringic acid were found in the fraction of hydroxybenzoic acids.

Low contents in flavan-3-ols were found in all samples, which can be explained in the low pressing used. Mayén et al. (1997) found similar values in white wines from *Pedro Ximenez* and *Baladi* grapes produced in the Montilla-Moriles region (southern Spain).

Phenolic composition can be modified by yeast during must fermentation as a result of conversion of non-phenolic compounds into phenolic compounds; transformation of high molecular weight polyphenols into others of low molecular weight; losses by precipitation and adsorption to yeast (Cuinier, 1988), and by the solubilisation and extraction of polyphenols by the ethanol generated during the process (Ghiselli et al., 1998). Guillén et al. (1997) observed that certain polyphenols, such as gallic acid, caftaric acid, *cis* and *trans p*-coutaric acids, protocatechuic acid, caffeic acid and *p*-coumaric acid exhibited significant increases during fermentation of sherry musts.

In our work, procyanidin B1, epicatechin, caftaric acid, *cis* and *trans p*-coutaric acid and caffeic acid concentrations increased during alcoholic fermentation. It could be explained by solubilisations and extractions favoured by the ethanol content.

Volatile Compounds

The major volatile compounds quantified were 2,3-butanediol, 2-methyl-1-butanol, isoamyl alcohol, ethyl acetate, 3-hydroxy-2-butanone, acetic acid, 2-phenylethanol, hexanoic acid, octanoic acid and isoamyl acetate (Table 3).

These results agreed with the values found in the literature (Mallouchos et al., 2002; Pozo-Bayón et al., 2002; Torija et al., 2002; Torrea et al., 2003). A high content in 2,3-butanediol was found in all samples. In wine, most of this compound is formed by yeasts during the fermentation of carbohydrates, with 3-hydroxy-2-butanone as an intermediate compound. Strains of the species of *Saccharomyces cerevisiae* which was used to inoculate our musts were reported as producing high amounts of 2,3-butanediol and only a few mg/L of 3-hydroxy-2-butanone (Romano et al.,

Table 2. Polyphenolic compounds from *Palomino fino* wine samples subjected to destemming. A: 2 days after inoculation. B: At the end of fermentation.

	Sample 1 (0%)		Sample 2 (25%)		Sample 3 (50%)		Sample 4 (75%)	
Compound (mg/L)	A	B	A	B	A	B	A	B
Hydroxybenzoic acids								
Gallic acid	5.19 ± 1.01	2.40 ± 0.18	3.70 ± 0.05	10.47 ± 1.30	8.32 ± 0.33	6.08 ± 0.54	6.69 ± 0.78	3.29 ± 0.34
Syringic acid	1.49 ± 0.05	1.44 ± 0.12	0.94 ± 0.34	1.76 ± 0.12	2.01 ± 0.13	1.20 ± 0.13	2.22 ± 0.09	1.82 ± 0.35
Hydroxycinnamic acids and esters								
Caftaric acid	30.69 ± 2.34	36.98 ± 1.45	22.73 ± 2.20	40.95 ± 3.15	30.30 ± 0.18	37.17 ± 1.98	33.16 ± 2.45	38.57 ± 3.14
2-S-glutathionyl caftaric acid	8.19 ± 1.34	7.38 ± 1.22	4.38 ± 0.87	10.12 ± 1.11	9.81 ± 1.10	8.36 ± 0.45	7.72 ± 1.32	8.53 ± 1.00
cis p-coutaric acid	2.98 ± 0.12	3.49 ± 0.58	1.84 ± 0.03	3.62 ± 0.48	3.37 ± 0.58	3.49 ± 0.34	3.94 ± 0.87	4.27 ± 0.65
trans p-coutaric acid	7.29 ± 0.43	9.08 ± 1.01	4.86 ± 0.65	9.49 ± 1.22	7.74 ± 1.02	9.24 ± 0.43	8.80 ± 0.98	10.10 ± 0.87
Fertaric acid	0.68 ± 0.12	0.65 ± 0.34	0.34 ± 0.07	0.65 ± 0.31	0.64 ± 0.12	0.64 ± 0.08	0.70 ± 0.02	0.86 ± 0.14
Caffeic acid	5.12 ± 1.00	5.09 ± 0.45	1.52 ± 0.23	2.82 ± 0.11	4.07 ± 1.02	4.27 ± 0.76	2.65 ± 0.43	5.00 ± 1.23
trans p-coumaric acid	0.72 ± 0.12	0.38 ± 0.06	0.39 ± 0.13	0.41 ± 0.05	0.72 ± 0.34	0.27 ± 0.12	1.25 ± 0.09	0.56 ± 0.22
Ferulic acid	0.41 ± 0.11	0.32 ± 0.04	0.24 ± 0.05	0.62 ± 0.16	0.37 ± 0.23	0.35 ± 0.16	0.51 ± 0.06	0.41 ± 0.17
Flavan-3-ols								
Procyanidin B1	0.91 ± 0.23	2.54 ± 0.32	1.36 ± 0.11	1.60 ± 0.10	1.83 ± 0.34	3.43 ± 0.56	2.20 ± 0.74	3.02 ± 0.87
Catechin	6.20 ± 1.10	3.81 ± 1.55	4.99 ± 1.23	1.53 ± 0.67	5.71 ± 1.34	4.26 ± 1.21	6.90 ± 0.65	4.63 ± 0.46
Procyanidin B2	5.80 ± 1.20	4.47 ± 0.99	5.98 ± 0.23	1.69 ± 0.05	7.72 ± 0.55	2.41 ± 0.14	8.64 ± 1.32	4.43 ± 0.05
Epicatechin	2.28 ± 0.34	2.05 ± 0.55	3.05 ± 0.76	3.68 ± 0.66	2.98 ± 0.44	3.76 ± 0.55	4.61 ± 1.00	6.38 ± 0.45

Table 3. Volatile compounds of samples *Palomino finowie* variety subjected to destemming. A: 2 days after inoculation. B: At the end of fermentation.

	Sample 1 (0%)		Sample 2 (25%)		Sample 3 (50%)		Sample 4 (75%)	
Compound* (mg/L)	A	B	A	B	A	B	A	B
Acids and esters								
Ethyl acetate	36.22 ± 2.18	50.29 ± 2.58	67.89 ± 3.44	48.97 ± 3.56	65.15 ± 2.20	40.72 ± 5.55	46.32 ± 3.14	38.13 ± 2.23
Ethyl butanoate	0.236 ± 0.005	0.146 ± 0.022	0.549 ± 0.123	0.081 ± 0.033	0.336 ± 0.076	0.090 ± 0.009	0.275 ± 0.103	0.075 ± 0.004
Ethyl pentanoate	nd	nd	nd	nd	nd	nd	nd	nd
Isoamyl acetate	2.04 ± 0.03	3.34 ± 0.34	5.54 ± 1.01[a]	3.82 ± 0.12	2.66 ± 0.78	1.89 ± 0.23	2.26 ± 0.12	2.92 ± 0.43
Hexyl acetate (μg/L)	78.1 ± 12.0	20.1 ± 10.2	309.2 ± 11.1	54.1 ± 8.3	336.0 ± 76.4	0.3 ± 0.2[a]	137.1 ± 34.1	0.1 ± 0.3[a]
cis-3-Hexenyl acetate	nd	nd	nd	nd	0.010 ± 0.007[a]	nd	0.008 ± 0.002[a]	nd
Ethyl lactate	nd	0.500 ± 0.010	nd	0.800 ± 0.034	11.27 ± 1.23	nd	15.02 ± 1.44	nd
Methyl octanoate (μg/L)	2.1 ± 1.1[a]	nd	0.02 ± 0.01[a]	nd	0.1 ± 0.1[a]	1.3 ± 0.1[a]	1.2 ± 0.1[a]	nd
Ethyl octanoate	0.853 ± 0.232	1.138 ± 0.322	1.953 ± 0.123	0.826 ± 0.034	1.483 ± 0.045	0.676 ± 0.067	1.036 ± 0.045	0.778 ± 0.056
Acetic acid	5.35 ± 0.45	13.01 ± 0.98	28.81 ± 2.23	28.14 ± 2.33	3.64 ± 0.66	9.21 ± 0.53	1.185 ± 0.41	29.60 ± 2.01
Methyl decanoate (μg/L)	nd	0.8 ± 0.2[a]	nd	nd	nd	0.2 ± 0.1[a]	nd	0.5 ± 0.1[a]
Ethyl 2-furoate	nd	nd	0.030 ± 0.010[a]	nd	nd	nd	nd	nd
Butanoic acid	nd	nd	0.274 ± 0.013	0.225 ± 0.034	0.046 ± 0.002	0.230 ± 0.005	0.057 ± 0.012	0.503 ± 0.100
Ethyl decanoate	0.035 ± 0.003	0.467 ± 0.078	0.328 ± 0.054	0.414 ± 0.088	0.176 ± 0.005	0.201 ± 0.013	0.210 ± 0.054	0.279 ± 0.032
Diethyl succinate	0.135 ± 0.008	0.375 ± 0.100	0.596 ± 0.018	0.396 ± 0.056	0.357 ± 0.043	0.112 ± 0.054	0.230 ± 0.078	0.016 ± 0.003[a]
Ethyl-2-phenyl acetate	nd	nd	nd	nd	nd	nd	nd	nd
Phenylethyl acetate	0.267 ± 0.078	0.481 ± 0.056	1.097 ± 0.121	0.822 ± 0.100	0.535 ± 0.123	0.376 ± 0.087	0.420 ± 0.023	0.587 ± 0.102
Hexanoic acid	1.607 ± 0.321	17.81 ± 1.34	7.66 ± 0.56	21.30 ± 1.78	5.89 ± 1.09	8.01 ± 0.78	5.50 ± 1.20	6.33 ± 1.56
Octanoic acid	2.78 ± 0.045	5.46 ± 1.10	3.99 ± 1.45	3.45 ± 0.76	2.76 ± 0.23	2.72 ± 0.45	2.62 ± 0.56	3.69 ± 0.44
Decanoic acid	0.886 ± 0.008	1.153 ± 0.011	1.304 ± 0.056	1.024 ± 0.102	0.992 ± 0.122	0.881 ± 0.122	1.224 ± 0.432	1.023 ± 0.122
Alcohols								
2-Methyl-1-butanol	99.83 ± 5.09	109.38 ± 6.12	215.80 ± 10.23	83.61 ± 11.09	53.46 ± 9.89	105.47 ± 10.11	35.77 ± 3.45	83.43 ± 7.87
Isoamyl alcohol	150.05 ± 12.11	132.55 ± 9.01	220.77 ± 8.98	107.13 ± 3.23	286.37 ± 10.98	90.13 ± 11.98	244.05 ± 13.23	98.43 ± 5.65
1-Hexanol	0.097 ± 0.001	0.175 ± 0.034	0.494 ± 0.065	0.207 ± 0.022	0.919 ± 0.103	0.403 ± 0.053	0.346 ± 0.006	0.212 ± 0.007
cis-3-Hexen-1-ol	0.002 ± 0.001[a]	nd	0.015 ± 0.002[a]	nd	nd	nd	nd	nd
2,3-Butanediol	289.39 ± 12.31	321.01 ± 5.22	295.01 ± 2.20	305.23 ± 11.20	301.78 ± 13.09	310.13 ± 10.21	332.01 ± 13.11	341.11 ± 7.01
Benzyl alcohol	nd	0.412 ± 0.012	nd	0.357 ± 0.021	nd	0.356 ± 0.023	nd	0.332 ± 0.009
2-Phenylethanol	7.79 ± 1.22	18.98 ± 2.43	24.21 ± 1.32	16.93 ± 1.22	14.70 ± 1.10	20.30 ± 2.33	11.18 ± 1.42	14.69 ± 1.00
Aldehydes and Ketones								
2-Furancarboxaldehyde	0.030 ± 0.007	0.238 ± 0.023	0.243 ± 0.022	nd	0.330 ± 0.044	nd	0.544 ± 0.041	nd
Benzaldehyde	nd	nd	0.023 ± 0.003[a]	nd	0.036 ± 0.008[a]	nd	0.035 ± 0.007[a]	nd
3-OH-2-butanone	35.99 ± 2.34	29.67 ± 2.35	67.56 ± 1.98	26.12 ± 0.34	54.70 ± 3.12	19.00 ± 1.10	17.68 ± 2.32	19.72 ± 2.12

*mg/L except for those specifically indicated. Nd: not detected.
[a] Values lower than LOQ.

1996). 2-Methyl-1-butanol, isoamyl alcohol, and 2-phenylethanol were produced mainly during the first stages of fermentation. Torija et al. (2002) found that 2-phenylethanol was produced in the early stages of the fermentation and that the concentration of medium-chain fatty acids increased sharply at the beginning of fermentation.

Among the esters identified, the major compounds were ethyl acetate, isoamyl acetate, 2-phenylethyl acetate, ethyl octanoate, diethyl succinate and ethyl decanoate. Torrea et al. (2003) found that the production of esters in musts from the Chardonnay variety took place during the fermentation from 50 to 75% of sugars because their formation is inhibited by the presence of oxygen (Jackson, 1994). However, Mauricio et al. (1993) observed that the hydrolysis-related esterase activity increased at the end of fermentation and provoked a decrease in their concentration. Previous data indicated that fatty acid esters production reached a maximum level followed by a decrease in their concentration (Vas et al., 1999; Moreno et al., 1995). Some of the enzymes involved in the synthesis of these esters are inhibited by high levels of ethanol (Malcorps and Dufour, 1992). The enzyme involved in the synthesis of ethyl acetate and isoamyl acetate has been shown to maintain its activity at higher ethanol levels than ethyl hexanoate synthase.

The content in esters of our musts increased steadily, reaching a maximum near the end of fermentation (6 days after the inoculation). Then, they slightly decreased (data not shown), probably due to the increasing of the esterase activity and/or the inhibition of the enzymes involved in their synthesis.

Among acids, butanoic, hexanoic and octanoic reached the highest concentrations. The synthesis of these acids begins from the beginning of the fermentation and continues through this period. A decrease in the levels of these compounds in the last phase of fermentation has also been reported by Fraile et al. (2000).

The highest concentrations for these compounds were observed at the fourth day of fermentation with a diminution at the end of this period, in agreement with the results of Fraile et al. (2000) and Herraiz et al. (1989). These losses could be attributed to absorptions in the cell walls and their utilisation by the yeasts.

Statistical Analysis

The samples considered here are a group defined by different levels of two factors: the degree of destemming (0, 25, 50 and 75%) and the process of fermentation (0, 2, 4, 6 and 9 days). Forty-two variables were considered, fourteen corresponding to polyphenolic compounds.

Analysis of Variance (ANOVA)

Two ANOVAs were carried out according to process of fermentation and destemming. Fisher's weight was calculated to establish the statistical significance of each factor on the compounds studied (Table 4).

The first factor, the process of fermentation, had a statistically significant effect on most of the compounds. Those compounds exhibiting the highest F values were, principally, volatile compounds, such as ethyl pentanoate, 2-phenylethanol, ethyl butanoate, 3-hydroxy-2-butanone and ethyl acetate.

Table 4 presents the results obtained from the study of comparison of means using the Tukey's test for both factors. As could be expected, for the 'fermentation' factor, most of the values corresponding to the volatile compounds before fermentation (0) were found to be significantly different from the other values (2, 4, 6 and 9 days). In some cases (ethyl butanoate, isoamyl acetate, hexyl acetate, 2-phenylethanol, benzyl alcohol and 3-hydroxy-2-butanone), these changes continue being significant throughout fermentation. As can be seen, the main changes in the volatile content are taking place during the first stages of fermentation.

Some polyphenols were also significantly affected by the fermentation factor (Table 4). These changes could be explained as being due to solubilisations, precipitations and adsorptions onto yeast (Cuinier, 1988; Guillén et al., 1997).

For the 'destemming' factor, the content of only certain polyphenolic compounds were a function of this factor at $p < 0.01$. These were, mainly, epicatechin, *trans* *p*-coumaric acid, *trans* and *cis* *p*-coutaric acids, caftaric acid, caffeic acid and syringic acid. No relationship between degree of destemming and changes in these compounds was observed. Musts obtained from destemmed grape did not exhibit clearly lower polyphenolic content. This could be explained by the short length of time that musts and stems were in contact.

In the case of volatile content, it can be deduced, from the results obtained, that the destemming did not modify the production of volatile compounds during fermentation.

Principal Component Analysis (PCA)

PCA is a good statistical tool to investigate associations between variables, moreover it is useful to detect natural groups among samples. If each sample is described by n variables, PCA creates a set of orthogonal axes that are linear combinations of the original variables. The first principal component retains the maximum variation among the data. Since our compounds were measured in different scales, we have used the variance–covariance matrix of standardised variables.

Table 4. Analysis of variance applied to samples from *Palomino fino* variety. Factors: destemming and alcoholic fermentation.

	Analysis of variance					
	Destemming		Fermentation		Differences (Tukey Test)	
Compounds	*F*	*p*	*F*	*p*	Destemming	Fermentation
Polyphenols						
Hydroxybenzoic acids						
Gallic acid	0.77	0.519	5.63	0.001[a]	–	0 vs. 6
Syringic acid	4.55	0.009[a]	8.97	0.000[a]	0% vs. 75%	0 vs. all
Hydroxycinnamic acids and esters						
Caftaric acid	5.88	0.002[a]	1.85	0.143	25% vs. 50%	–
GRP	4.27	0.012	2.32	0.078	25% vs. 50%	–
cis p-coutaric acid	5.08	0.005[a]	6.18	0.000[a]	50% vs. 75%	0 vs. 6 & 9
trans p-coutaric acid	5.92	0.002[a]	4.59	0.005[a]	25% vs. 50%	0 vs. 9
Fertaric acid	2.56	0.071	0.93	0.459	–	–
Caffeic acid	4.89	0.006[a]	6.36	0.001[a]	0% vs. 25%	0 vs. 6 & 9
trans p-coumaric acid	8.05	0.000[a]	6.43	0.001[a]	75% vs. 25% & 50%	2 vs. 9
Ferulic acid	1.02	0.397	21.76	0.000[a]	–	0 vs. all
Flavan-3-ols						
Procyanidin B1	2.05	0.126	9.50	0.000[a]	–	0 vs. all
Catechin	1.17	0.336	23.79	0.000[a]	–	0 vs. 2, 4 & 6; 9 vs.2, 4 & 6
Procyanidin B2	1.64	0.197	6.60	0.001[a]	–	0 vs. 9
Epicatechin	20.94	0.000[a]	0.07	0.990	75% vs. all	–
Volatile compounds						
Acids and esters						
Ethyl acetate	0.53	0.666	30.79	0.000[a]	–	0 vs. all
Ethyl butanoate	0.17	0.914	39.43	0.000[a]	–	0 vs. 2, 4 & 6; 9 vs. 2, 4 & 6
Ethyl pentanoate	–	–	–	–	–	–
Isoamyl acetate	2.09	0.120	22.46	0.000[a]	–	0 vs. all; 2 vs. 6
Hexyl acetate	0.78	0.512	26.42	0.000[a]	–	0 vs. 2, 4 & 6; 9 vs. 2, 4 & 6
cis-3-Hexenyl acetate	1.34	0.276	12.44	0.000[a]	–	2 vs. all
Ethyl lactate	1.98	0.136	2.01	0.116	–	–
Methyl octanoate	1.68	0.190	0.62	0.650	–	–
Ethyl octanoate	0.69	0.566	21.93	0.000[a]	–	0 vs. all
Acetic acid	4.99	0.006[a]	2.25	0.084	25% vs. 50%	–
Methyl decanoate	1.15	0.343	3.05	0.031	–	–
Ethyl 2-furoate	1.29	0.292	3.18	0.026	–	–
Butanoic acid	2.16	0.111	5.91	0.001[a]	–	0 vs. 9
Ethyl decanoate	2.33	0.092	11.26	0.000[a]	–	0 vs. 4, 6 & 9
Diethyl succinate	2.17	0.109	8.21	0.000[a]	–	0 vs. all
Ethyl-2-phenyl acetate	–	–	–	–	–	–
Phenylethyl acetate	3.41	0.028	13.63	0.000[a]	–	0 vs. 4, 6 & 9
Hexanoic acid	2.35	0.090	5.51	0.002[a]	–	0 vs. 9
Octanoic acid	2.62	0.066	9.68	0.000[a]	–	0 vs. 4, 6 & 9
Decanoic acid	1.58	0.212	8.64	0.000[a]	–	0 vs. all
Alcohols						
2-Methyl-1-butanol	0.31	0.816	9.84	0.000[a]	–	0 vs. 4 & 6
Isoamyl alcohol	0.25	0.862	9.98	0.000[a]	–	0 vs. 2, 4 & 6
1-Hexanol	4.14	0.013	8.08	0.000[a]	–	0 vs. 9
cis-3-Hexen-1-ol	2.02	0.129	1.40	0.255	–	–
2,3 Butanediol	1.71	0.183	6.15	0.001[a]	–	2 vs. 6
Benzyl alcohol	3.04	0.042	4.65	0.003[a]	–	9 vs. 2 & 6
2-Phenylethanol	0.36	0.780	59.24	0.000[a]	–	0 vs. all; 2 vs. 4 & 6; 9 vs. 6
Aldehydes and ketones						
3-Hydroxy-2-butanone	0.48	0.695	35.31	0.000[a]	–	0 vs. all; 9 vs.4 & 6
2-Furancarboxaldehyde	0.40	0.756	4.59	0.005[a]	–	0 vs. 2
Benzaldehyde	1.38	0.266	1.40	0.256	–	–

[a] Values are significantly different at $p < 0.01$.
0, 2, 4, 6 and 9 days after inoculation.

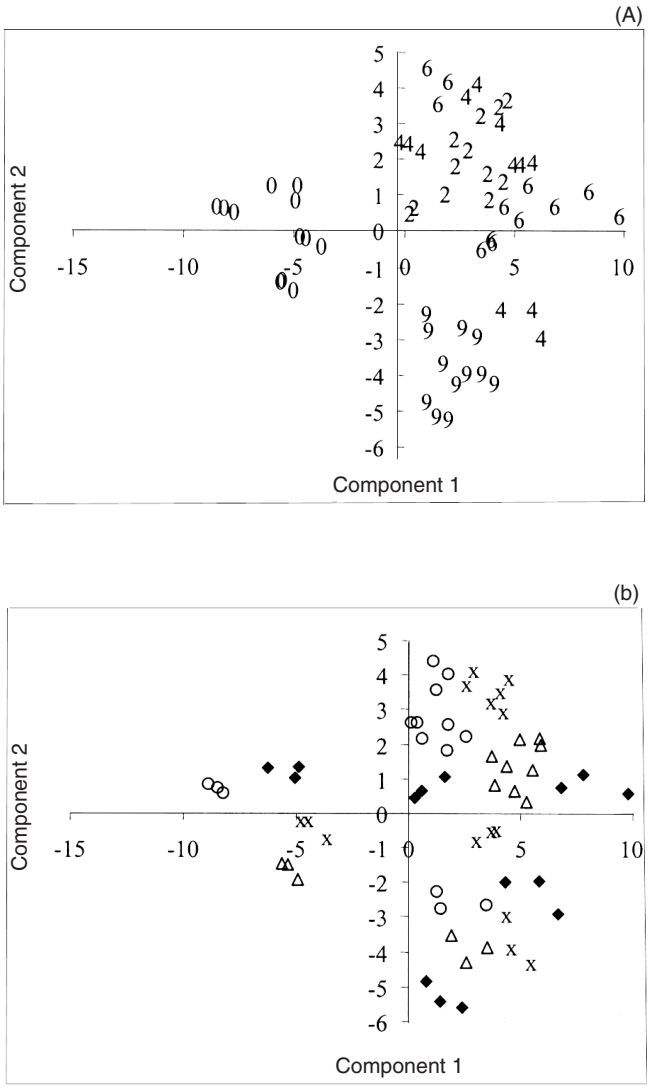

Figure 1. PCA. Plot of the first two principal components. (a): 0, 2, 4, 6 and 9 days after inoculation. (b) Percent of destemming: 0 (◆) , 25 (△), 50 (○) and 75% (x).

When the data matrix (polyphenols and volatile compounds) was subjected to PCA, 10 significant PCs emerged according to Kraiser´s criterion (eingenvalues >1). With these factors, 88.01% of total variance is explained. The first PC, component 1, which explained 35.94% of total variance, mainly contains ethyl acetate, isoamyl acetate, ethyl octanoate, phenylethyl acetate and 2-phenylethanol. Constituents of component 2 were benzyl alcohol, caftaric acid, hexyl acetate, 1-hexanol and catechin. As can be seen, these two PCs are most highly correlated with volatile compounds.

The first component differentiated between samples before and after fermentation (Figure 1a). Component 2, which explains 12.65% of total variance, separated samples corresponding to the start of fermentation from those corresponding to the end of this process (9 days). No differentiation according to degree of destemming was found (Figure 1b).

The distribution obtained corroborated that grape destemming did not modify the production of volatile compounds during fermentation.

From the results obtained, it can be concluded that musts from destemmed grapes of the *Palomino fino* variety produced wines with similar polyphenolic content to those obtained without destemming.

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REFERENCES

Aleixandre J.L., Casp A. and García M.J. (1998). Effects of vinification technique on Alto Turia (D.O. Valencia) white wine composition. *Rivista di Viticoltura e di Enologia* **51**: 19–29.

Bréchot P., Chauvet J., Dupuy P., Croson M. and Rabatu A. (1971). Acide olénolique, facteur de croissance anaérobie de la levure de vin. *CR Academic Science Service D* **272**: 890–893.

Cantarelli C. (1989). Phenolics and yeast: remarks concerning fermented beverages. *Yeast* **5**: 53–61.

Casas J. (1985). Descripción resumida de la técnica enológica de los vinos de Jerez. In: *Proceedings of the University of Cadiz, III Jornadas Universitarias sobre el Jerez*. Cádiz, Spain: Jiménez-Mena, Artes Gráficas, pp. 333–361.

Castino M. (1981). Conseguenze sul colore dei vino rossi della macerazione prensenza di raspi. *Rivista di Viticoltura e di Enologia* **12**: 547–551.

Castro R., Natera R., Benítez P. and Barroso C.G. (2004). Comparative analysis of volatile compounds of fino sherry wine by rotatory and continuous liquid-liquid extraction and SPME in conjunction with GC-MS. *Analytica Chimica Acta* **513**: 141–150.

Christaki T. (2002). Quality and safety assurance in winemaking. *Food Control* **13**: 503–517.

Cuinier C. (1988). Influence des levures sur les composés phénoliques du vin. *Bulletin de l 'OIV* **61**: 596–601.

Fraile P., Garrido J. and Ancín C. (2000). Influence of a *saccharomyces cerevisiae* selected strain in the volatile composition of rose wines: evolution during fermentation. *Journal of Agricultural and Food Chemistry* **48**: 1789–1798.

Ghiselli A., Nardini M., Baldi A. and Scaccini C. (1998). Antioxidant activity of different phenolic fractions separated from an Italian red wine. *Journal of Agricultural and Food Chemistry* **46**: 361–367.

Guillén D.A., Barroso C.G. and Pérez-Bustamante J.A.

(1997). Automated solid phase extraction by HPLC-DAD for the monitoring of phenolic compounds during fermentation of sherry must. *Química Analítica* **16**: 21–25.

Herraiz T., Tabera J., Reglero G., Martín-Alvarez P.J., Herraiz M. (1989). Production of short- and medium-chain length alcohols and fatty acids during ethanol fermentation with *Saccharomyces cerevisiae*. *Belgian Journal of Food Chemistry and Biotechnology* **44**: 88–94.

Jackson R.S. (1994). *Wine Sciences: Principles and Applications.* San Diego: Academic Press Inc., pp. 203–223.

Macheix J.J., Fleuriet A. and Billot J. (1990). *Fruit phenolics*. Boca Raton: CRC Press Inc., pp. 323–332.

Macheix J.J., Sapis J.C. and Fleuriet A. (1991). Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. *Critical Reviews in Food Science and Nutrition* **30**: 441–486.

Malcorps P. and Dufour J. (1992). Short-chain and medium-chain aliphatic-ester synthesis in *Saccharomyces cerevisiae. European Journal of Biochemistry* **210**: 1015–1022.

Mallouchos M., Komaitis A., Koutinas M. and Kanellaki J. (2002). Investigations of volatiles evolution during the alcoholic fermentation of grape must using free and immobilized cells with the help of solid phase microextraction (SPME) headspace sampling. *Journal of Agricultural and Food Chemistry* **50**: 3840–3848.

Mauricio J.C., Moreno J.J., Valero E.M., Zea L., Medina M. and Ortega J.M. (1993). Ester formation and specific activities of in vitro alcohol acetyltransferase and esterase by *Saccharomyces cerevisiae* during grape must fermentation. *Journal of Agricultural and Food Chemistry* **41**: 2086–2091.

Mayén M., Barón R., Mérida J. and Medina M. (1997). Changes in phenolic compounds during accelerated browning in white wines from cv. Pedro Ximenez and cv. Baladi grapes. *Food Chemistry* **58**: 89–95.

Moreno J., Bravo M., Mauricio J.C., Ortega J.M. and Medina M. (1995). Production of higher alcohols and their acetates under different fermentation conditions by *Saccharomyces cerevisiae*. *Acta Horticulturae* **388**: 239–245.

Pozo-Bayón M.A., Pueyo E., Martín-Alvarez P.J. and Polo M.C. (2002). Polydimethylsiloxane solid-phase microextraction-gas chromatography method for the analysis of volatile compounds in wines: its application to the characterization of varietal wines. *Journal of Chromatography A* **922**: 267–275.

Rapp A. and Mandery H. (1986). Wine Aroma. *Experientia* **42**: 873–884.

Revilla E., Alonso E. and Kovac V. (1997). The content of catechins and procyanidins in grapes and wines as affected by agroecological factors and technological practices. *ACS Symposium Services* **661**: 69–80.

Romano P. and Suzzi, G. (1996). Minireview: origin and production of acetoin during wine yeast fermentation. *Applied and Environmental Microbiology* **62**: 309–315.

Schreier P. (1979). Flavor composition of wines: a review. *Critical Reviews in Food Science and Nutrition* **12**: 59–111.

Singleton V.L. (1987). Oxygen with phenols and related reactions in musts, wines, and model systems: observations and practical implications. *American Journal of Enology and Viticulture* **38**: 69–77.

Singleton V.L., Timberlake C.F. and Lea A.G.H. (1978). The phenolic cinnamates of white grapes and wine. *Journal of the Science of Food and Agriculture* **29**: 403–410.

Souquet J.M., Labarbe B., LeGuerneve C., Cheynier V. and Moutounet M. (2000). Phenolic composition of grape stems. *Journal of Agricultural and Food Chemistry* **48**: 1076–1080.

Sun B.H., Spranger I., Roque do Valle F., Leandro C. and Belchior P. (2001). Effect of different winemaking on phenolic composition in Tinta Miuda red wines. *Journal of Agricultural and Food Chemistry* **49**: 5809–5816.

Torija M.J., Beltrán G., Novo M., Poblet M., Guillamón J.M., Mas A. and Rozès N. (2002). Effects of fermentation temperature and *Saccharomyces* species on the cell fatty acid composition and presence of volatile compounds in wine. *International Journal of Food Microbiology* **85**: 127–136.

Torrea D., Fraile P., Garde T. and Ancín C. (2003). Production of volatile compounds in the fermentation of Chardonnay musts inoculated with two strains of *Saccharomyces cerevisiae* with different nitrogen demands. *Food Control* **14:** 565–571.

Vas G., Blechschmidt I., Kovacs T. and Vekey K. (1999). Examination of aroma production kinetics of different commercial wine yeast in fermenting muscat ottonel wines with the help of SPME headspace sampling and fast GC analysis. *Acta Alimentaria* **28**: 133–140.