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Influence of Prefermentative Treatments to the Major Volatile Compounds of Assyrtiko Wines

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A study of the volatile fraction of Assyrtiko wines, using gas chromatography coupled with olfactometry, was realized. Twenty-seven volatile compounds were identified as potent odorants, most of them originating from the fermentation process. Quantification of the major volatile compounds was realized developing a rapid analytical method based on fractionation of a 50 mL wine aliquot using C_{18}-reversed phase adsorbent. After elution of the volatile compounds with pentane−diethyl ether and concentration under nitrogen, the final wine extract was injected in a gas chromatography−flame ionization detection system. The method allows satisfactory determination of more than 15 volatile compounds of wine. The linearity of the method gave a typical r^2 between 0.990 and 0.999, while reproducibility ranged from 5.1 to 12.2% (as relative standard deviation) with 9.5% as the average. The method was applied to wines produced by Assyrtiko grapes (AOC Santorini), for two consecutive years, to compare the effect of skin contact prior to fermentation and the must clarification process. Direct press and skin contact wines were differentiated analytically; however, highly significant differences were not. Inversely, the differences found between direct press/clarified and nonclarified wines were significant. Wines produced by direct press and clarified must presented significantly higher levels of ethylic esters and fusel alcohol acetates but lower fusel alcohol levels, leading probably to more fruity wines. This difference, between clarified and nonclarified grape musts, was not significant in the case of the wines produced by skin contact of Assyrtiko berries. These findings were validated by preference sensory analysis tests.

KEYWORDS: Wine; aroma; aromatic compounds; Assyrtiko; prefermentative skin maceration; decanting

INTRODUCTION

Over 1000 volatile compounds have been identified, with a wide concentration range varying between hundreds of mg/L to ng/L (*1*). Moreover, wine aroma is generated by several classes of compounds such as alcohols, esters, organic/volatile acids, aldehydes, ketones, lactones, sulfur, nitrogen compounds, and terpenes. Their combination and their levels differentiate one wine from another (*1*–*3*).

The estimation of food impact aroma could be performed by various olfactometric techniques, as discussed recently by Pollien et al. (*4*). To characterize each wine, flavor chemists used gas chromatography coupled with olfactometry (GCO). GCO seems to be the most appropriate technique, because the human senses and analytical apparatus are combined to complement the available detection capabilities (*5*–*7*).

The great number of volatile components identified and the fact that they have a different chemical nature covering a wide range of polarity, solubility, volatility, and pH explain the difficulty of quantitative analysis of these compounds. The ideal volatile compounds quantification method would be that reported by Guth (*8*) who, by using isotopomers, complex sample preparation protocols, and numerous gas chromatography−mass spectrometry (GC-MS) runs, was able to accurately quantify 43 wine odor-active compounds. Quantification of wine major volatile compounds could probably be realized using less complex analytical procedures. A single GC−flame ionization detection (FID) chromatogram from a wine or grape extract can provide quantitative data on compounds formed during wine making (*9*). There are several methods described in the literature that partially fulfill these requisites. Liquid−liquid extraction continues being the reference technique for the extraction of volatile components from wine (*10*). Dynamic headspace techniques have been applied for studying the aromatic composition of wine recently (*11*). More recently, solid-phase microextraction (SPME) was reported for varietal characteriza-

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Table 1. Method Linearity Data and Calibration Graphs

compound	intercept	slope	r^2	range (mg/L)
ethyl isobutyrate	0.0117	0.3444	0.9994	0.15−1.1
ethyl butyrate	43.857	21.882	0.9914	0.15−1.1
ethyl-2-methylbutyrate	−0.062	0.4903	0.99	0.15−1.1
ethyl hexanoate	0.02	0.5120	0.99	0.5−3.5
ethyl octanoate	0.0185	0.4951	0.9962	0.75−3.5
isobutyl acetate	0.0135	0.3706	0.9989	0.15−1.1
isoamyl acetate	0.0003	0.4382	0.9954	0.75−3.5
2-phenylethyl acetate	0.0819	11.695	0.9919	0.15−1.1
amylalcohol	−0.0619	0.5816	0.9973	0.75−3.5
2-methyl-1-propanol	0.0982	0.1848	0.9924	0.75−3.5
2-/3-methyl-1-butanol	0.5996	0.4337	0.9922	0.75−35
hexanol	−0.0062	0.6133	0.9962	0.75−3.5
cis-3-hexen-1-ol	0.0407	0.527	0.9998	0.75−3.5
3-methylthio-1-propanol	0.0184	0.051	0.9989	0.75−3.5
geraniol	0.1252	10.735	0.997	0.15−1.1
2-phenylethanol	12.244	0.8153	0.9957	0.75−35

tion of wines and analysis of the wine bouquet using different fibers (*12, 13*).

Solid-phase extraction using XAD-2 resin (*14*) was also used for the quantification of wine volatile compounds. This method has the important advantage that someone can isolate and analyze both free and bound volatile compounds (*14*).

V. vinifera L. cv. Assyrtiko is considered to be the most interesting Greek white grape, originating from the island of Santorini. It is well-adapted on the volcanic ground of the island of Santorini and its special climatic conditions (*15*). The major volatile compounds characterizing the aroma of the wines of this variety have not been identified in any previous studies. Thus, the first target of the present work was to identify the potent odorant compounds and then to develop an analytical method able to quantify the major volatile compounds in a single chromatographic run. The method was then applied to study the influence of prefermentative techniques on these compounds, to examine which of these techniques would be more appropriate for the production of high-quality wines. These prefermentation techniques are skin maceration in low temperatures and monitored must clarification to different levels of grape solids. Skin maceration of crushed white grapes prior to pressing has commonly been used recently as the flavor components are extracted from the skins (*16*). It is also known that the quality of white wines is improved by lowering insoluble solids levels in the juice prior to fermentation. Studies of juice on wine quality (*17–19*) indicated that wines prepared from clarified juice were higher in wine quality. Also, it was reported (*20*) that in the presence of grape solids, the formation of higher alcohol was elevated. The present work aims to investigate the effect of prefermentative methods on the major aroma compounds of Assyrtiko wines.

MATERIAL AND METHODS

Chemicals. *n*-Pentane was purchased from Merck (99.8%), diethyl ether and tartaric acid (99.5%) were from Riedel-de Haen, and methanol (99.5%) was from Laboratory Scan. Water was purified through a cation exchange column from Ionel, solid anhydrous sodium sulfate (99%) was purchased from Panreac, sodium hydroxide was from J.T . Baker, and C_{18} cartridges (tube size, 20 mL, 5 g) were from Resprep. Octan-3-ol (1 g/L in absolute ethanol) was used as an internal standard. Exact masses (1 g/L) of the chemical standard compounds were dissolved in absolute ethanol to be used as standard solutions of the analytes. The volatile compounds (**Table 1** or **2**) were purchased by Fluka, Riedel-de Haen, Acros Organics, and Aldrich, and the purity for all of the standards was more superior than 98%.

Wine Samples. All wine samples were from Santorini, and the experiment was realized for two consecutive vintages of 2004 and 2005.

Table 2. Relative Standard Deviation (RSD %) at Concentrations Higher than the Quantification Limit (LOQ)

volatile compound	RSD %[a]	LOD (mg/L)[b]	LOQ (mg/L)[c]
ethyl isobutyrate	10.2	0.005	0.01
ethyl butyrate	8.4	0.02	0.05
ethyl-2-methylbutyrate	8.0	0.01	0.03
ethyl hexanoate	8.0	0.01	0.03
ethyl octanoate	8.7	0.01	0.03
isobutyl acetate	10.0	0.08	0.15
isoamyl acetate	10.4	0.08	0.15
2-phenylethyl acetate	10.6	0.1	0.3
amylalcohol	10.6	0.1	0.2
2-methyl-1-propanol	10.0	0.1	0.2
2-/3-methyl-1-butanol	10.9	0.1	0.2
hexanol	11.0	0.3	0.5
cis-3-hexen-1-ol	10.2	0.01	0.03
3-methylthio-1-propanol	11.1	0.3	0.5
geraniol	12.2	0.2	0.2
2-phenylethanol	5.1	0.3	0.5

[a] RSD %, relative standard deviation. [b] LOD (mg l^{-1}): detection limit, 3× signal/noise. [c] LOQ (mg l^{-1}): quantification limit, 5× signal/noise.

Grapes of the Assyrtiko variety were harvested at an industrial ripeness stage (between 12th and 15th of August), corresponding to wines containing approximately 12% ethanol for both of the vintages. The samples were processed (destemming and crushing) separately to obtain two batches of must, one by direct pressing of the grapes (sample code, DP) and the other originating from the berries subjected to skin maceration at 10−12 °C for 12 h (sample code, SC). Potassium metabisulfite was added to the grapes prior to pressing adjusting the total SO_2 to 60 mg/L. These two different kinds of musts were further separated in two different fermentation conditions: One included fermentation (after direct pressing or after skin maceration) without solids (clarified grape juice; sample code, DD), and the other included fermentation (after direct pressing or after skin maceration) "with grape solids" (nonclarified grape juice; sample code, NDD). Clarification was realized by gravity-induced sedimentation at 10−12 °C for 12 h. The conditions mentioned above were confirmed by measurement of the NTU value of all four conditions. The "clean" musts had an NTU value of around 50, while the musts fermenting "with grape solids" had an NTU value of around 1000.

All of the musts were inoculated with 0.25 g/L of commercial *Saccharomyces cerevisiae* yeast stain (UVAFERM 228). Fermentations were conducted by using 30 L inox tanks that were kept at a temperature between 16 and 18 °C throughout. All macerations and fermentations were performed in triplicate.

Analysis of ethanol, reducing sugars, absorption at 420 nm, volatile acidity, total acidity, pH, and total phenolics (absorbance at 280 nm) were determined using OIV (*21*) official methods. The nephelometric turbidity units (NTU) were measured using a Hach 2100P portable turbidity meter (Beavercreek, OH).

Synthetic Wine Samples. Standard solutions were diluted with water and alcohol (adjusting the final alcohol content to 12%, v/v) at concentrations typically found in wine. All solutions were added with 5 g/L tartaric acid, and the pH was adjusted to 3.2 with 1 M NaOH.

Isolation of Volatiles from Wines for GCO. Two hundred milliliters of wine was poured in a 0.5 L Erlenmeyer and cooled to 1 °C in an ice bath under nitrogen. Dichloromethane (40 mL) was added, and the mixture was stirred for 15 min at 700 rpm (*22*). The wine−solvent mixture was supplemented with 40 mL of dichloromethane, and stirring was continued for 15 min. The organic phase was separated in a separatory funnel, centrifuged for 5 min at 10000*g* (4 °C), dried over sodium sulfate, and then concentrated by distillation through a Rotavapor down to 0.4 mL and then under a nitrogen stream (N_2 5.0 quality). The final concentration factor was 500.

GCO Analysis. GCO analysis was carried out using a Fison Instruments gas chromatograph GC8060, fitted with a 60 m fused-silica column (0.32 mm i.d. and 0.25 μm film thickness), coated either with DB Wax (J&W Scientific) or with DB 5 (J&W Scientific Folsom, CA). The injection (2 μL) of the extract was splitless/split (split ratio, 1/20)

in an injection port heated to 230 °C. The carrier gas was helium (5.0 quality), with a flow rate of 2 mL/min. The oven temperature program was 40 °C (for 3 min), then increased at 4 °C/min to 85 °C, and held at this temperature for 3 min, then increased at 3 °C/min up to 230 °C (or 250 °C in the case of DB-5), and held at this temperature for a further 20 min. The GC effluents were split to a sniffing port and a flame ionization detector (3/1). The detector temperature was set at 230 °C. The dilution factors (FD) of the identified wine volatiles were estimated, as reported by Guth (*6*). The wine extract was stepwise diluted with dichoromethane 1:5, 1:25, and 1:625; then, 2 μL of each dilution was injected into the GCO system; and the sniffing tests were performed by two trained persons. Only the odors smelled by both of the trained persons were retained for estimating the FD of each volatile compound.

GC-MS Analysis. GC-MS analysis was performed on a Fisons 8000 series gas chromatograph (model 8060) coupled to a Fisons MD-800 quadrupole mass spectrometer. Helium was used as the carrier gas (2.0 mL/min). The separation of compounds was performed on 60 m fused-silica column (0.32 mm i.d. and 0.25 μm film thickness), coated with DB Wax (J&W Scientific). The oven temperature program was the same as reported previously for the GCO analysis. The injector, ion source, and interface temperatures were set at 230, 200, and 250 °C, respectively. Electron impact mass spectra were recorded at 70 eV of ionization energy in the 29−400 *m/z* mass range. Identification of compounds was done by comparing the retention times and MS data with those of standard compounds and by MS data obtained from Wiley and NIST libraries.

GC for Quantitative Analysis. A Hewlett-Packard 5890 Series II gas chromatograph was used. The column (30 m × 0.32 mm and 0.25 mm film thickness) was a DB Wax from J&W Scientific. The temperature program was as follows: 40 °C for 5 min and then raised at 3 °C/min up to 230 °C. The carrier gas was helium at 1 mL/min. Injection was 1 μL in split mode. The split flow was 70.6 mL/min. Detection was achieved by FID.

Proposed Analytical Method. Each cartridge of C_{18} reversed-phase (tube size, 20 mL, 5 g of phase) was preconditioned with 20 mL of methanol and then with 20 mL of Milli-Q water. Afterward, 50 mL of wine supplemented with 10 μL of internal standard, octanol-3 (final concentration in the sample 5 mg/L), and solution was loaded onto the C_{18} RP cartridge at a flow rate of ca. 3 mL/min, and then, the cartridge was washed with 75 mL of Milli-Q water while all of the above extracts were disposed. The final extract of the 20 mL of pentane−diethyl ether (1:1 v/v) solvent was recovered in a 40 mL vial. Any water residues at the pentane−diethyl ether extract were removed by the addition of the necessary amount of Na_2SO_4. The extract was concentrated down to 1 mL under N_2 and transferred to a 2 mL vial. Then, it was injected into the gas chromatograph under the conditions listed above. The relative response areas for each of individual wine volatile compounds to the internal standard were calculated (see **Table 1**) and interpolated in the corresponding calibration graphs built as described below, to express volatile compounds concentrations at mg/L.

Calibration Graphs. Synthetic wines containing known amounts of the volatile compounds, 12% (v/v) ethanol, 5 g/L tartaric acid, and pH adjusted to 3.2 with 1 M sodium hydroxide were extracted and analyzed following the proposed procedure. The range of concentrations tested can be seen in **Table 1** (five concentrations for each compound).

Repeatability. One Assyrtiko wine, 2004 vintage, was spiked with known amounts of wine volatile compounds and with 10 μL of the internal standard solution and extracted as indicated in the proposed method five times during the same day. The relative areas of analytes to the internal standard were calculated. The repeatability was estimated as RSD = [SD (relative area $_{1-5}$)/average (relative area $_{1-5}$)] × 100. The repeatability data are shown in **Table 2**.

RESULTS AND DISCUSSION

GCO Results. Table 3 represents the potent odorant compounds, which were identified in Assyrtiko wine samples by GCO and GC-MS. Most of the identified compounds are considered as secondary metabolites of alcoholic fermentation.

Table 3. Impact Odorants of Assyrtiko Wines

	RI		FD factor	
volatile compounds	DB WAX	DB 5	Assyrtiko extract	descriptor
ethanal	750	<600	1	apple/fruit
ethyl isobutyrate	929	757	25	fruity/pineapple
diacetyle	987	600	1	butter/yogurt
isobutyl acetate	1005	860	1	fruity/green
ethyl butyrate	1023	804	25	strawberry
ethyl 2-methylbutyrate	1077	849	25	fruity/apple
2-methyl-1-propanol	1105	647	5	nail varnish
isoamyl acetate	1143	880	25	banana
2-/3-methyl-1-butanol	1192	736	625	nail varnish
ethyl hexanoate	1227	1000	25	apple
hexanol	1360	872	1	grass
cis-3-hexen-1-ol	1400	858	1	grass
ethyl octanoate	1436	1200	25	pear
acetic acid	1449	628	25	vinegar
3-(methylthio)propanal	1474	905	25	baked potato
linalool	1555	1103	1	muscat
2-methylpropanoic acid	1565	775	5	cheese
butanoic acid	1615	829	25	cheese
phenylethanal	1625	1047	25	honey
2-/3-methylbutanoic acids	1661	868	625	parmesan cheese
3-methylthio-1-propanol	1715	982	25	raw potato
2-phenylethyl acetate	1808	1260	25	rose
β-damascenone	1820	1395	25	canned apple
hexanoic acid	1841	1017	5	grass/fruity
geraniol	1852	1120	1	floral
2-phenylethanol	1902	1116	625	rose
furaneol	2043	1062	25	caramel

Ethyl esters of straight chain fatty acids, ramified esters, acetates of higher alcohols, higher alcohols, volatile acids, lactones, aldehydes, ketones, and sulfur compounds were identified, all of them formed by yeast metabolism (*1*). Besides, terpenols and one C_{13} nor-isoprenoid were identified, compounds that are considered to have their origin directly from the grapes (*1*). While caution should be applied in interpreting GCO data sets and relations with the wine aroma, overall, these results could be a helpful direction for characterization of the aroma of Assyrtiko wines.

From the class of terpenols, linalool and geraniol have been identified, described by the panelists as Muscat/floral. According to many authors, terpenes (*1, 2*) have been found to play an important role in the aroma composition of Muscat-derived wines. However, in the case of Assyrtiko wines, they could probably participate but moderately, as the FD factor found is very low (**Table 3**). In any case, Muscat-like descriptors are not associated with the Assyrtiko aroma (*15*). β-Damascenone occurs widely in grapes and many fruits contributing to their aroma with its fruity, fruity marmalade, honey/cooked apple note (*1, 23*). This compound belongs to the C_{13} nor-isoprenoid class. β-Damascenone is believed to originate from the breakdown of the carotenoid neoxanthin by a complex pathway (*1*). The Assyrtiko wine extract seems to exhibit high FD factors; however, this also does not mean that it participates importantly to the aroma of these wines. The very low detection threshold in olfactometry for this compound explains why it is found at the highest dilution factor in aroma extract dilution analysis methods (*23*). β-Damascenone is characterized by a very low perception threshold in a model wine solution, but in a wine, the threshold was found to be over 1000-fold higher (*23*).

Ethyl esters of isobutyric, butyric, 2-methylbutyric, hexanoic, and octanoic acids were found to be the compounds that are predominantly responsible for the fruity flavor of Assyrtiko wine since most of these compounds exhibited relatively high FD

factors (FD = 25, **Table 3**). The aroma of these ethyl esters was described as apple, pineapple, and pear.

Three fusel alcohols, 2-methyl-1-propanol, 2-/3-methyl-1-butanol, and 2-phenylethanol (**Table 3**), displayed high FD factors. These compounds are well-known wine aroma compounds (*1*), although their contribution to the wine aroma is not obvious. 2-Methyl-1-propanol and 2-/3-methyl-1-butanol presented odors described as nail varnish, while 2-phenylethanol was described as roselike. 2-/3-Methyl-1-butanol and 2-phenylethanol were found to be among the three compounds exhibiting the highest FD factor (625). Both of these compounds generally present the highest concentrations among the volatile compounds of the wines in numerous varieties (*1, 2*). Furthermore, their acetates, isobutyl, isoamyl, and phenylethyl, were identified in the Assyrtiko wine extracts, and especially, isoamyl and phenylethyl acetate presented relatively high FD factors. Two of three (isobutyl- and isoamyl-) were described as fruity. Isoamyl acetate presented characteristic banana-like aroma, while 2-phenylethyl acetate exhibited a roselike aroma similar to that of its corresponding fusel alcohol.

Acetic, 2-methylpropanoic, butanoic, 2-/3-methylbutanoic, and hexanoic acids showed high FD factors and could contribute to wine aroma as their levels in wines are generally high (*1*). Acetic acid, a characteristic compound of vinegars, showed a FD factor of 25; however, it is rather difficult that this compound could participate importantly in the aroma of Assyrtiko wines, as its perception threshold is very high (*8*). All of the other acids, with the exception of hexanoic acid, exhibited strong cheesy aromas, and especially, the mixture of 2- and 3-methylbutanoic acids was the third compound presenting the highest FD factor (= 625), among the potent odorant compounds identified in Assyrtiko wine extracts. Hexanoic acid was described as green/fruity; however, the fruity hints should be due to the compounds eluting before, of β-damascenone, when using the polar capillary column and of ethyl hexanoate when using the nonpolar one. Despite the high levels for these volatile compounds found in wines and their high FD factors, it is rather difficult that they contribute to the wine aroma (*8*). It was proposed that their contribution should be weak because of the high threshold value found for these acids in water/ethanol media (*8*).

Among the identified potent odorant compounds were hexanol and *cis*-3-hexen-1-ol with their green grass notes, exhibiting low FD factors, however. Ethanal and diacetyle, both of them highly volatile compounds, were identified in the extract also exhibiting low FD- factors. Phenylethanal, described as honeylike, presented a high FD factor and could probably participate in the aroma of Assyrtiko wines. Two sulfur compounds were also identified and described as baked and raw potato, 3-(methylthio)-propanal and 3-methylthio-1-propanol, respectively. Both of these compounds present low olfactory thresholds and probably participate in the wine aroma. 3-Methylthio-1-propanol was mentioned to have a very low olfactory threshold of 0.2 ng/L in water; therefore, tasters could recognize the potato-like smell in very low concentrations (*24*). Finally, Furaneol was also identified, presenting a caramel-like odor. This compound was found to differentiate the Merlot from Cabernet Sauvignon wines (*25*).

In summary, numerous potent odorants are responsible for the overall flavor impression of Assyrtiko wines. The GCO analysis results provide an overall view of aroma compounds in the wines of this variety; 27 potent odorants were identified. Notably, the odor active compounds found in this work were those called major volatile compounds, also identified in the wines of other varieties as well. Besides these major odor active compounds, the Assyrtiko wine was characterized by additional nice fruity, flowery, and sweetlike notes of isoamyl acetate and ethyl esters such as ethyl butyrate, ethyl 2-methylbutyrate, and ethyl hexanoate. However, not very attractive aromas were also identified, as those of cheese and raw potato.

Method Validation. Among the 27 identified potent odorants, only some of them could be quantified. Ethanal and diacetyle were not possible to be quantified because of their high volatility and their coelution with solvents. Also, other aldehydes found generally in traces in the wines such as phenylethanal and 3-methylthio-propanal were not evident to be quantified. Compounds such as linalool and β-damascenone were not quantified as their levels in non-Muscat varieties are very low (*1*). Furaneol needs a stable isotope dilution assay and thus GC-MS to be quantified accurately (*26*). Quantification of the volatile acids identified in the extracts was attempted, but correlation coefficients (r^2) ranged from 0.96 to 0.97, and their RSDs were disappointing. The reason is that the 75 mL of water used for washing the cartridges flashed down these acidic compounds; thus, repeatability was very poor. In conclusion, by using this method, volatile acids and compounds with acidic properties cannot be quantified. However, these compounds do not contribute to the wine aroma importantly (*8*); thus, despite the disadvantage of the method, it could be used for quantification of the major wine compounds. Inversely, one compound, not revealed by sniffing, amylalcool, was quantified as being present in all of the extracts. **Table 1** summarizes method linearity data for the rest of the compounds as found in synthetic model wine solutions. Five calibration graphs were built for each compound. Data in the table clearly show that linearity is satisfactory in almost all of the cases, with the coefficient of correlation (r^2) ranging from 0.99 (ethyl hexanoate) to 0.9998 (*cis*-3-hexen-1-ol). Linearity holds at least for 1 order of magnitude, and in most cases, it holds for at least two, which ensures that the normal concentration range of nearly all of the compounds present in **Table 1** is comprised in the linear range of the method. The slope of the straight calibration lines is a measure of method sensitivity and depends on both extraction efficiency and detector response for each compound.

Repeatability data are given in **Table 2**. Repeatability is the average standard deviation of a mean obtained from several replicate samples analyzed in the same batch. **Table 4** shows that for synthetic alcoholic solutions, the RSD varies from 5.1 (for 2-phenylethanol) to 12.2% (for geraniol) for the synthetic solutions, with an average value of 9.5% for the synthetic wines, which can be considered satisfactory for the purpose of the analysis.

In conclusion, the proposed method allows for fast and clean quantitative determination of more than 15 volatiles in wine. Among these volatiles are some important analytes, markers for the microbiological state of wine, for the wine sensory characteristics, or the wine origin (both geographic and varietal) and oak barrel origin. The analytical characteristics—linearity, precision, and accuracy—of the method are satisfactory. All of these characteristics make the method useful for wine quality control and classification and able to give information that could be used in the control of winemaking processes.

Basic Chemical Composition of the Wines. The basic chemical compositions of the wines obtained with different prefermentative treatments in 2004 and 2005 vintage are given in **Tables 4** and **5**, respectively. In 2004, vintage wines produced by skin contact (SCDD and SCNDD) presented higher total phenolics and browning indices (absorbance at 420 nm), which

Table 4. General Chemical Composition of *V. vinifera* L. cv. Assyrtiko Wines, Vintage 2004[a]

	vintage 2004			
general chemical analysis	DPDD	DPNDD	SCDD	SCNDD
alcohol % (v/v)	11.1 b ± 0.1	10.9 b ± 0.2	11.6 a ± 0.2	11.2 b ± 0.1
total acidity (g/L tartaric acid)	7.2 b ± 0.2	7.7 a ± 0.2	7.2 b ± 0.2	8.1 a ± 0.3
pH	2.90 a ± 0.2	2.95 a ± 0.2	2.77 b ± 0.4	2.70 b ± 0.3
volatile acidity (g/L acidic acid)	0.4 a ± 0.1	0.15 b ± 0.1	0.5 a ± 0.1	0.6 a ± 0.1
total phenolics	11.2 c ± 1.00	11.5 c ± 0.7	12.8 b ± 0.6	13.9 a ± 0.5
absorbance at 420 nm	0.119 d ± 0.03	0.126 c ± 0.02	0.136 b ± 0.03	0.175 a ± 0.08

[a] Standard deviations are calculated taking into account the average of three analyses of the three different wines corresponding to each fermentation. Values followed by the same letter do not show statistical differences at 5%. DPDD, direct press clarified grape juice; DPNDD, direct press nonclarified grape juice; SCDD, skin contact clarified grape juice; and SCNDD, skin contact nonclarified grape juice.

Table 5. General Chemical Composition of *V. vinifera* L. cv. Assyrtiko Wines, Vintage 2005[a]

	vintage 2005			
general chemical analysis	DPDD	DPNDD	SCDD	SCNDD
alcohol % (v/v)	12.2 b ± 0.1	12.9 a ± 0.3	12.5 b ± 0.3	12.3 b ± 0.3
total acidity (g/L tartaric acid)	8.1 b ± 0.2	8.5 a ± 0.1	7.8 b ± 0.3	8.00 b ± 0.2
pH	3.05 a ± 0.2	3.2 a ± 0.2	2.90 a ± 0.2	2.85 a ± 0.2
volatile acidity (g/L acidic acid)	0.28 a ± 0.08	0.27 a ± 0.06	0.32 a ± 0.05	0.27 a ± 0.1
total phenolics	15.5 b ± 0.7	18.6 a ± 0.7	14.9 b ± 0.5	15.1 b ± 0.7
absorbance at 420 nm	0.077 c ± 0.03	0.095 a ± 0.03	0.083 b ± 0.05	0.085 b ± 0.05

[a] Standard deviations are calculated taking into account the average of three analyses of the three different wines corresponding to each fermentation. Values followed by the same letter do not show statistical differences at 5%. DPDD, direct press clarified grape juice; DPNDD, direct press non clarified grape juice; SCDD, skin contact clarified grape juice; and SCNDD, skin contact non clarified grape juice.

signifies that a dissolution of phenolic compounds was realized during the maceration, in accordance with previous works (*27, 28*). Treated and untreated with skin maceration wines presented almost similar values for alcohol content and volatile acidity. Total acidity values were not lower in the case of skin macerated samples as expected, and pH values were lower. Using skin contact usually leads to lower total acidity and higher pH because of the liberation of potassium from the skins and thus partial salification of tartaric acid (*28*). Reducing sugars were found to be inferior to 2 g/L in all samples.

Additionally, differences were observed between the wines produced with (DD) or without total solids (NDD). In both of the cases, DP (direct press) and SC (skin contact) total acidity was higher for the nonclarified juices. This is probably explained by the fact that decanting leads to a reduction of total acidity as tartaric acid precipitates during this procedure (*29*). However, nonsignificant change was observed for the pH values between the samples and for alcohol levels and the same trend was found for volatile acidity. Finally, a higher content of total phenolics (even though nonsignificant for the direct press samples) and higher browning indices were found for the nonclarified samples, which is rather logical as one of the advantages of decanting prior to fermentation is that of leading to white wines with more attractive color (*29*). Reducing sugars were found to be inferior to 2 g/L in all samples.

Different trends were revealed for 2005 vintage wines, at least when comparing direct press and skin contact samples. Skin contact wines (SCDD and SCNDD) did not present higher total phenolics. The same was observed for the browning indices (absorbance at 420 nm). The difference was not found for alcohol content and volatile acidity—both presented values very close between the treated and the untreated with skin maceration wines. Total acidity and pH values were not lower and higher, respectively, in the case of skin macerated samples, as expected. However, in the comparison between the samples produced with (DD) or without total solids (NDD), some differences were observed. In DP (direct press) samples, the total acidity was found to be higher for the nonclarified juices, similar to that found for the samples of 2004 vintage. In the case of SC sample, nonsignificant differences were revealed. Nonsignificant changes were observed for the pH values between the samples and for alcohol levels and for volatile acidity. A higher content of total phenolics, for the direct press samples, was found, and the same was found for browning indices. The differences were nonsignificant for the SC samples.

Globally, according to the results found in this experiment, skin contact did not significantly result in wines with higher total phenolics and browning indices. For 2004 vintage, the wine composition seemed to be affected by the skin maceration, but the same trend was not followed for 2005 vintage wines. Concerning clarified and nonclarified samples, a trend of higher total acidities was revealed, even though nonsignificant for all of the cases. The same was revealed for total phenolics.

Prefermentation Treatments Influence on Major Volatile Compounds. The proposed analytical method was applied on the analysis of Assyrtiko wines produced by different winemaking procedures. A comparison of Assyrtiko wines produced by classical white vinification and by prefermentative skin maceration was realized, as also by clarification or not, to study the influence of this method for the production of Assyrtiko wines. The chemical groups of compounds analyzed were those of ethylic esters, fusel alcohols, and their acetates, volatile acids, 3-methylthio-propanol, C_6 alcohols, and geraniol.

In the 2004 vintage (**Table 6**), ethylic esters were significantly higher in the case of direct press wines. Only in the case of ethyl isobutyrate, skin contact produced wines and presented higher levels; however, this quantification is characterized as tentative, as the values found for ethyl isobutyrate are above the linear range tested during this assay. Also, total ethylic esters levels were found to be higher for the direct press sample. These findings are opposite to that reported previously (*27, 28*). Fusel alcohols were found to be significantly higher in skin contact wines. C_6 alcohols were found to be significantly higher in the skin contact wines; however, the difference between direct press nonclarified wines and the two skin contact wines was not significant. The same trends were also observed by previous

Table 6. Effect of Skin Contact and Decanting on the Major Aroma Compounds of *V. vinifera* L. cv. Assyrtiko Wines, Vintage 2004[a]

	2004			
compound	DPDD	DPNDD	SCDD	SCNDD
ethyl isobutyrate[b]	0.09 b ± 0.01	0.04 c ± 0.01	0.13 a ± 0.01	0.13 a ± 0.02
ethyl butyrate	1.16 a ± 0.12	0.56 c ± 0.08	0.98 b ± 0.09	0.48 c ± 0.04
ethyl-2-methylbutyrate	0.20 a ± 0.01	0.13 b ± 0.02[b]	0.15 b ± 0.01	0.14 b ± 0.01[b]
ethyl hexanoate	0.78 a ± 0.08	0.46 b ± 0.04[b]	0.59 b ± 0.06	0.45 b ± 0.06[b]
ethyl octanoate[b]	0.67 a ± 0.08	0.44 b ± 0.05	0.54 b ± 0.06	0.40 b ± 0.07
sum ethylic esters	2.90 a ± 0.08	1.64 c ± 0.07	2.48 b ± 0.07	1.57 c ± 0.05
isobutyl acetate	tr[c]	tr[c]	0.21 a ± 0.02[b]	0.17 a ± 0.03[b]
isoamyl acetate	0.51 c ± 0.04[b]	0.24 d ± 0.04[b]	1.01 a ± 0.07	0.62 b ± 0.03[b]
2-phenylethyl acetate	tr[c]	tr[c]	tr[c]	ND[d]
sum of acetates	0.82 b ± 0.04	0.41 c ± 0.06	1.41 a ± 0.04	0.84 b ± 0.04
amylalcohol	tr[c]	tr[c]	tr[c]	tr[c]
2-methyl-propanol	18.34 a ± 0.81	21.20 a ± 0.83	20.74 a ± 1.80	20.10 a ± 1.56
2-/3-methyl-butanol	245.31 c ± 4.61	280.99 b ± 6.63	337.22 a ± 13.4	307.93 a ± 11.08
sum of fusel alcohols	263.78 c ± 5.43	301.99 b ± 7.46	358.08 a ± 15.23	328.17 a ± 12.60
cis-3-hexen-1-ol[b]	0.06 b ± 0.01	0.1 a ± 0.01	0.1 a ± 0.01	0.13 a ± 0.03
hexanol	0.75 b ± 0.05	1.18 a ± 0.1	1.33 a ± 0.1	1.15 a ± 0.05
sum of C_6 alcohols	0.81 b ± 0.05	1.28 a ± 0.11	1.44 a ± 0.1	1.29 a ± 0.07
3-methylthio-propanol	1.12 b ± 0.06	1.73 a ± 0.06	1.69 a ± 0.01	1.72 a ± 0.12
geraniol	1.23 a ± 0.06	0.91 b ± 0.06	1.12 a ± 0.06	0.56 c ± 0.04
2-phenylethanol	34.02 b ± 0.82	26.36 c ± 0.83	42.81 a ± 1.30	40.92 a ± 0.74

[a] Results expressed in mg/L. Standard deviations are calculated taking into account the average of three analyses of the three different wines corresponding to each fermentation. Values followed by the same letter do not show statistical differences at 5%. DPDD, direct press clarified grape juice; DPNDD, direct press nonclarified grape juice; SCDD, skin contact clarified grape juice; and SCNDD, skin contact nonclarified grape juice. [b] Tentative quantification: The values are above the linear range tested but higher than the LOQ. [c] tr, trace. Values found are lower than the LOQ found for the compound. [d] ND, not detected. Values found are lower than LOD found for the compound.

studies (*27, 28, 30*). 3-Methylthio-1-propanol levels were significantly higher in skin contact wines, while geraniol levels were found to be almost equal between the direct press and the skin contact wines. Phenyl ethanol levels were significantly higher for the skin contact wines following the trend found for the other fusel alcohols. When comparing clarified and nonclarified samples, significant differences were found especially for the direct press wines, while between the clarified and the nonclarified wines originating from skin contact of the berries, the differences were not significant. In detail, ethylic esters levels were higher in the clarified direct press wines. The same results were found for isoamyl acetate also (even though the values found should be considered as tentative); thus, decanting of the must probably leads to more fruity wines as was already reported by previous works using the grapes of other varieties (*31, 32*). Furthermore, fusel alcohols, hexanol, and 3-methylthio-1-propanol levels were significantly higher in the case of the nonclarified sample while 2-phenylethanol and geraniol levels were significantly higher in the case of the clarified sample. It was reported that a low level of turbidity was needed to ensure a complete fermentation as well as ester formation. It has also been reported that large quantities of sediment in the juice not only caused off odor but also retarded the production of esters by the yeast. They concluded that the ester content is probably low in the wines from unsettled juices with high levels of insoluble solids. However, in the case of skin contact wines, nonsignificant differences were found for ethylic esters with the exception of ethyl butyrate levels. Also, isoamyl acetate levels were found to be significantly higher in the clarified sample. Despite that, these compounds participate importantly to the fruity aroma of wines; the results found suggest that nonclarified wines are not presenting higher ethylic esters levels in comparison to the clarified sample. Fusel alcohols, hexanol, 3-methylthio-1-propanol, and 2-phenylethanol levels were not significantly higher in the case of the nonclarified sample. Only geraniol levels were found to be significantly higher in the case of the clarified sample.

In the 2005 vintage (**Table 7**), ethylic esters levels between direct press and skin contact wines were not significantly higher. The same trends were found for acetates levels, despite the fact that fusel alcohols levels were significantly higher in direct press samples. Hexanol was found to be significantly higher in the skin contact wines. The same trends were also observed by previous studies (*27, 28, 30*). This seems logical, as this compound could be considered as varietal originating from the wax cuticule of the skins. Skin contact processing facilitates the extraction of these compounds, as the grape juice is in contact with the skins for 12 h. *cis*-3-Hexen-l-ol possesses an herbaceous, leafy odor (its common name is leaf alcohol) and was found to increase with extraction during skin contact of Chardonnay grapes at cool temperatures (around 10 °C, close to the temperatures of our experiment). Hexanol, which smells only slightly herbaceous, displayed the same behavior (*16*). These six-carbon compounds related to leafy or herbaceous aromas develop during skin contact. 3-Methylthio-1-propanol levels were significantly higher in the direct press wines, inverse of that found for 2004 wines, while geraniol levels were found to be almost equal between the direct press and the skin contact wines, following the trend found for 2004 wines. 2-Phenyl ethanol levels were significantly higher for the direct press clarified wines, while no difference was found between the nonclarified direct press and the clarified skin contact wines.

Table 7. Effect of Skin Contact and Decanting on the Major Aroma Compounds of *V. vinifera* L. cv. Assyrtiko Wines, Vintage 2005[a]

	2005			
compound	DPDD	DPNDD	SCDD	SCNDD
ethyl isobutyrate[b]	0.05 a ± 0.01	0.05 a ± 0.00	0.04 a ± 0.01	0.06 a ± 0.01
ethyl butyrate	0.90 a ± 0.06	0.48 b ± 0.08	1.04 a ± 0.12	0.72 b ± 0.07
ethyl-2-methylbutyrate	0.19 a ± 0.02	0.13 b ± 0.02[b]	0.15 b ± 0.01	0.16 b ± 0.01
ethyl hexanoate	0.68 a ± 0.12	0.53 a ± 0.05	0.72 a ± 0.13	0.61 a ± 0.03
ethyl octanoate[b]	0.67 a ± 0.08	0.40 b ± 0.03	0.42 b ± 0.03	0.49 b ± 0.06
sum of ethylic esters	2.69 a ± 0.06	1.77 c ± 0.03	2.54 b ± 0.09	2.23 b ± 0.11
isobutyl acetate	0.28 a ± 0.02	0.18 b ± 0.02	tr[c]	0.15 b ± 0.03
isoamyl acetate	1.18 a ± 0.08	0.85 b ± 0.04	1.13 a ± 0.10	0.79 b ± 0.05
2-phenylethyl acetate	0.43 a ± 0.04	0.28 b ± 0.02	0.20 b ± 0.03	0.21 b ± 0.03
sum of acetates	1.90 a ± 0.08	1.31 b ± 0.09	1.45 b ± 0.12	1.20 b ± 0.07
amylalcohol	tr[c]	tr[c]	tr[c]	tr[c]
2-methyl-propanol	11.49 b ± 0.82	20.93 a ± 1.25	14.17 b ± 0.85	12.66 b ± 1.20
2-/3-methyl-butanol	290.75 b ± 5.53	390.66 a ± 5.63	252.63 c ± 8.6	249.60 c ± 9.23
sum of fusel alcohols	302.4 b ± 5.78	411.56 a ± 6.46	266.96 c ± 14.37	262.43 c ± 11.34
cis-3-hexen-1-ol[b]	0.06 b ± 0.01	0.14 a ± 0.03	0.06 b ± 0.01	0.07 b ± 0.03
hexanol	0.93 c ± 0.05	1.29 b ± 0.15	1.92 a ± 0.20	1.69 a ± 0.12
sum of C_6 alcohols	0.99 c ± 0.05	1.42 b ± 0.10	1.98 a ± 0.1	1.76 a ± 0.12
3-methylthio-propanol	3.73 a ± 0.45	3.93 a ± 0.76	1.99 b ± 0.87	1.68 b ± 0.62
geraniol	0.69 a ± 0.06	0.52 b ± 0.08	0.74 a ± 0.09	0.65 a ± 0.06
2-phenylethanol	63.16 a ± 4.1	47.75 b ± 2.83	43.61 b ± 1.23	33.38 c ± 2.02

[a] Results expressed in mg/L. Standard deviations are calculated taking into account the average of three analyses of the three different wines corresponding to each fermentation. Values followed by the same letter do not show statistical differences at 5%. DPDD, direct press clarified grape juice; DPNDD, direct press nonclarified grape juice; SCDD, skin contact clarified grape juice; and SCNDD, skin contact nonclarified grape juice. [b] Tentative quantification: The values are above the linear range tested but higher than the LOQ. [c] tr, trace. Values found are lower than the LOQ found for the compound.

Table 8. Paired Comparison Test for Wines Made from Direct Press Clarified, Direct Press Nonclarified, Skin Contact Clarified, and Skin Contact Nonclarified Juices, Vintages 2004 and 2005[a]

	no. of samples preferred by tasters	
	paired sample	aroma
Assyrtiko 2004	DPDD vs DPNDD	15 vs 4[b]
	SCDD vs SCNDD	13 vs 8
	DPDD vs SCDD	12 vs 9
	DPNDD vs SCNDD	14 vs 3[b]
Assyrtiko 2005	DPDD vs DPNDD	12 vs 5[b]
	SCDD vs SCNDD	12 vs 8
	DPDD vs SCDD	13 vs 8
	DPNDD vs SCNDD	13 vs 7

[a] DPDD, direct press clarified grape juice; DPNDD, direct press non clarified grape juice; SCDD, skin contact clarified grape juice; and SCNDD, skin contact non clarified grape juice. [b] Significant at the 5% level.

Direct press clarified wines presented significantly higher levels of ethylic esters in comparison to nonclarified wines. Only ethyl isobutyrate levels (tentative quantification) were not found to be different; also, for hexanoate levels, a slight supremacy of clarified wines was found, however nonsignificant. In the case of skin contact wines, ethyl esters levels between clarified and nonclarified wines were not significant with the exception of ethyl butyrate levels, higher in the clarified wines, following the trend found for 2004 vintage. Isoamyl acetate levels were significantly higher in the clarified samples for both direct press and skin contact wines. Fusel alcohols levels were significantly higher in the nonclarified samples for the direct press wines but not for skin contact wines. Fusel alcohols positively affect the wine aroma in quantities less than 400 mg/L but negatively in higher quantities (*29*). The fusel alcohol content of the wines is influenced by juice clarification. Previous studies (*33, 34*) have demonstrated that suspended solids increased the formation of higher alcohols in wines, as also shown that musts containing more grape solids tend to produce wines with higher amounts of isobutyl, active amyl, and isoamyl alcohol than wines from settled and enzyme-treated juices. C_6 alcohols were found to be significantly higher in the nonclarified direct press wines, while for skin contact wines, any difference was not found between clarified and nonclarified wines. Phenyl ethanol levels were significantly higher for the direct press and skin contact clarified wines, in comparison to corresponding nonclarified wines.

In conclusion, quantification of these compounds, originating generally from alcoholic fermentation, in Assyrtiko wines produced by direct press or skin contact procedures, did not show a clearly significant difference for one or the other method of vinification. Only C_6 alcohols levels were significantly higher for the prefermentative skin maceration wines, in both of the vintages. Inversely, it was found that decanting, especially in the direct press wines, could lead to fruitier wines as ethylic esters and fusel alcohol acetates levels were significantly higher, and on the other hand, fusel alcohol levels, contributing generally negatively to the wine aroma, were found to be significantly lower. Noteworthy was the finding that in skin contact wines significant differences were not found between clarified and nonclarified wines, with the exception of ethyl butyrate and isoamyl acetate (significantly higher in the clarified samples, 2005 vintage). This could suggest that after skin contact of Assyrtiko grapes, a severe and time-consuming decanting is probably not necessary and the next white vinification steps could follow, earning time and protection toward exposure to O_2.

Sensory Evaluation of the Wine Samples. Direct press and skin contact wines and also clarified and nonclarified wines of both vintages were evaluated using triangle and preference tests (*35*). The wines were evaluated by a taste panel consisting of six to 10 judges with tasting experience (**Table 8**). For each pair of samples, there was a total of three tasting sessions. At each session, three pairs of wines were presented in different orders to each panelist in coded dark glasses at a serving temperature of 16 °C. The judge was asked to find the different one in the triangle tests and to express a preference between two wines in each pair. A total of 15−25 trials were conducted for each pair of wines. For each vintage, four pairs of samples were presented to the judges. Results were compared to the minimum numbers of agreeing judgments necessary to establish significant preference at various probability levels (two-tailed tests; $p = 1/2$).

The sample produced with direct pressing and decanting, DPDD, was compared to the direct press nonclarified one, DPNDD, and to the skin contact clarified one, SCDD. The sample produced with skin contact and decanting, SCDD, was compared to the nonclarified corresponding one, SCNDD, and the direct press nonclarified, DPNDD, to the skin contact nonclarified one, SCNDD. The samples were found to be different by the judges for both of the vintages ($p < 0.05$), but in the preference tests, significant results were only found between direct press clarified and nonclarified samples (in both of the vintages) and between direct press nonclarified and skin contact nonclarified only for the 2004 vintage. Any significant result was not found when comparing direct press and skin contact wines, neither between the two skin contact conditions. These findings mean that the wines produced by different postharvest technologies were differentiated by the judges, but the tasters could give a significant preference only in the case of direct press clarified wines. Thus, a juice originating from skin contact procedures could avoid obligatory subjection to severe clarification processes.

However, the wines from clarified juice were described by the panelists as fruity but having less varietal flavor. For the wines from nonclarified juices, the aroma was found to be less fruity. These results are correlated with the major volatile analysis results, where it was found that the clarified juices produced wines with higher ethylic esters and fusel alcohols acetates levels and besides less fusel alcohol levels.

LITERATURE CITED

(1) Etievant, P. X. Wine. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 483−533.

(2) Rapp, A. Volatile flavour of wine: Correlation between instrumental analysis and sensory perception. *Nahrung* **1998**, *42*, 351–363.

(3) Marti, M. P.; Mestres, M.; Sala, C.; Busto, O.; Guasch, J. Solidphase microextraction and gas-chromatography olfactometry analysis of successively diluted samples. A new approach of the aroma extract dilution analysis applied to the characterization of wine aroma. *J. Agric. Food Chem.* **2003**, *51*, 7861–7865.

(4) Pollien, P.; Ott, A.; Montigon, F.; Baumgartner, M.; Munoz- Box, R.; Chaintreau, A. Hyphenated headspace-gas chromatography-sniffing technique: screening of impact odorants and quantitative aromagram comparisons. *J. Agric. Food Chem.* **1997**, *45*, 2630–2637.

(5) Kotseridis, Y.; Baumes, R. Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation and in the produced wine. *J. Agric. Food Chem.* **2000**, *48*, 400–406.

(6) Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3022–3026.

(7) Komes, D.; Ulrich, D.; Lovric, T. Characterization of odor-active compounds in Croatian Rhine Riesling wine, subregion Zagorje. *Eur. Food Res. Technol.* **2006**, *222*, 1–7.

(8) Guth, H. Quantification and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032.

(9) Ortega, C.; Lopez, R.; Cacho, J.; Ferreira, V. Fast analysis of important wine volatile compounds. Development and validation of a new method based on gas-chromatographic-flame ionization detection analysis of dichloromethane microextracts. *J. Chromatogr. A* **2001**, *923*, 205–214.

(10) Villen, J.; Senorans, F. J. D.; Reglero, G.; Herraiz, M. Analysis of wine aroma by direct injection in gas chromatography without previous extraction. *J. Agric. Food Chem.* **1995**, *43*, 717–722.

(11) Garcia-Jares, C.; Garcia-Martin, S.; Cela-Torrijo, R. Analysis of some highly volatile compounds of wine by means of purge and cold trapping injector capillary gas chromatography. Application to the differentiation of Rias Baixas Spanish white wines. *J. Agric. Food Chem.* **1995**, *43*, 764–768.

(12) Whiton, R. S.; Zoecklein, B. W. Optimization of headspace solid-phase microextraction for analysis of wine aroma compounds. *Am. J. Enol. Vitic.* **2000**, *51*, 379–382.

(13) Pozo-Bayon, M. A.; Pueyo, E.; Martin-Alvarez, P. J.; Polo, M. C. Polydimethysiloxane solid-phase microextraction-gas chromatography method for the analysis of volatile compounds in wines: Its application to the characterization of varietal wines. *J. Chromatogr. A* **2001**, *922*, 267–275.

(14) Günata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes. Extraction and deterrmination of free and glycosidically bound fractions of some grape aroma compounds. *J. Chromatogr. A* **1985**, *331*, 83–90.

(15) Chapa, R.; Fallis, C.; Farrel, P. *The Global Encyclopedia of Wine*; Forrestal, P., Ed.; Wine Appreciation Guild: San Francisco, 2001; p 213.

(16) Ramey, D.; Bertrand, A.; Ough, C. S.; Singleton, V. L.; Sanders, E. Effects of skin contact temperature on chardonnay must and wine composition. *Am. J. Enol. Vitic.* **1986**, *37*, 99–106.

(17) Singleton, V. L.; Seiberhagen, H. A.; de Wet, P.; van Wyk, C. J. Composition and sensory qualities of wines prepared from white grapes by fermentation with and without grape solids. *Am. J. Enol. Vitic.* **1975**, *26*, 62–69.

(18) van Wyk, C. J. *The Influence of Juice Clarification on Composition and Quality of Wines*; Proceedings of the International Enology Symposium, Auckland, New Zealand, 1978; pp 33−45.

(19) Williams, J. T.; Ough, C. S.; Berg, H. W. White wine composition and quality as influenced by method of must clarification. *Am. J. Enol. Vitic.* **1978**, *29*, 92–96.

(20) Klingshirn, L. M.; Liu, J. R.; Gallanger, J. F. Higher alcohol formation in wines as related to the particle size profiles of juice insoluble solids. *Am. J. Enol. Vitic.* **1987**, *38*, 207–210.

(21) Recueil, O. I. V. *Des Méthodes Internationales d'Analyse des Vins et des Moûts*; Office Internationa de la Vigne et du Vin.: París, France, 1990.

(22) Moio, L.; Chambellaut, E.; Lesschaeve, I.; Issanchou, S.; Schlich, P.; Etievant, P. X. Production of representative wine extracts for chemical and olfactory analysis. *Ital. J. Food Sci.* **1995**, *3*, 265–278.

(23) Pineau, B.; Barbe, J. C.; Van Leeuen, C.; Dubourdieu, D. Which impact for β-damascenone on red wines aroma. *J. Agric. Food Chem.* **2007**, *55*, 4103–4108.

(24) Valim, M. F.; Rouseff, R. L.; Lin, J. Gas chromatographic olfactometry characterization of aroma compounds in two types of cashew apple nectar. *J. Agric. Food Chem.* **2003**, *51*, 1010–1015.

(25) Kotseridis, Y.; Razungles, A.; Bertrand, A.; Baumes, R. Differentiation of the aromas of Merlot and Cabernet Sauvignon wines using sensory and instrumental analysis. *J. Agric. Food Chem.* **2000**, *48*, 5383–5388.

(26) Schneider, R.; Kotseridis, Y.; Belancic Majcenovi, C. A.; Augier, C.; Razungles, A. Quantification of furaneol and homofuraneol in Vitis vinifera wines by stable isotope dilution assay using GC-

MS/MS. In *Flavour Research at the Dawn of the Twenty-First Century*; Le Quéré, J. L., Etiévant, P. X., Eds.; Tec. et Doc. Lavoisier: Cachan, France, 2003; pp 710−714.

(27) Selli, S.; Canbas, A.; Cabaroglu, T.; Erten, H.; Gunata, Z. Aroma components of cv. Muscat of Bornova wines and influence of skin contact treatment. *Food Chem*. **2006**, *94*, 319–326.

(28) Cabaroglou, T.; Canbas, A. Effects of skin contact on aromatic composition of the white wine of vinifera V. cv. L. Muscat of Alexandria grown in Southern Anatolia. *Acta Aliment*. **2002**, *31*, 45–55.

(29) Ribereau-Gayon, P.; Dubourdieu, D.; Doneche, B.; Lonvaud, A. The microbiology of wine and vinifications. In *Handbook of Enology*; J. Wiley & Sons: Chisester, 2000; pp 378−379.

(30) Symeou, E.; Galiotou-Panayotou, M.; Kechagia, D.; Kotseridis, Y. Analysis of major aroma compounds of Assyrtiko wines subjected to pre-fermentative skin maceration. *J. Agric. Sci*. **2007**, *145*, 577–585.

(31) Houtman, A. C.; Du Plessis, C. S. The effect of juice clarity and and several conditions promoting yeast growth on fermentation rate, the production of aroma components and wine quality. *S. Afr. J. Enol. Vitic*. **1981**, *2*, 71–82.

(32) Houtman, A. C.; Marais, J.; Du Plessis, *Vitis* **1980**, *19*, 37–54.

(33) Groat, M.; Ough, C. S. Effects of insoluble solids added to clarified musts on fermentation rate, wine composition, and wine quality. *Am. J. Enol. Vitic*. **1978**, *29*, 112–119.

(34) Ribereau-Gayon, P.; Lafon-Lafourcade, S.; Bertrand, A. Le dèbourbage de moûts de vendange blanche. *Connaiss. Vigne Vin* **1975**, *9*, 117–139.

(35) Larmond, E. *Méthodes d'Appréciation Sensorielle des Aliments*; Ministère de l'Agriculture du Canada: Ottawa, Canada, 1969.

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