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Influence of harvesting techniques, grape crushing and wine treatments on the volatile components of white wines

by

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Influence des techniques de vendange, de foulage et du traitement des vins sur les composants volatils de vins blancs

Résumé: Des raisins blancs du cépage Trebbiano ont été récoltés soit à la main, soit mécaniquement. Les raisins récoltés mécaniquement ont été foulés sur le champ et laissés tels quels ou bien introduits en atmosphère d'oxygène ou anhydridre sulfureux.

Après la vinification effectuée de manière traditionnelle les vins ont été stabilisés avec différents clarifiants. Ensuite, on a étudié les composants volatils des vins ainsi obtenus.

La methodologie mise au point comprend l'extraction avec solvent, la concentration de l'extrait et l'identification au moyen de la CPG et CPG/SM. De cette façon on a pu identifier une soixantaine de composants. En général on a observé que la récolte des raisins influe sur très peu de composants volatils et ceci seulement du point de vue quantitatif.

Le traitement de hypéroxygénation du foulage a causé d'une part un applâtissement du profil gaschromatographique, d'autre part il a stimulé la formation de molécules oxydées. L'apport d'anhydride sulfureux, au contraire, a augmenté les phénomènes de macération mais diminué la stabilité des vins à la reaction de brunissement.

L'influence des traitements stabilisants à la bentonite et aux autres clarifiants, a provoqué une diminution plus au moins accentuée des substances volatiles probablement à cause de leur volatilisation au cours des traitements.

Key words: grape harvest, technique, fining, wine, flavour, oxidation, extraction, stabilization.

Introduction

New problems in wine-making technology arise from the shaking of white grapes in mechanical harvesting. First of all, the breakage of fruit on their separation from the stem increases juice leak during transportation (16). This results both in undesirable biological activities and in oxidation and maceration of the product. To avoid this, the picked grapes are crushed directly in the vineyard and sulfur dioxide is added, or transported in inert gas atmosphere (4). The use of SO_2 in this phase inhibits the activity of oxidative enzymes and microorganisms, but enhances the polyphenol extraction.

To lower the SO_2 content and improve the white wine stability, a technique of programmed early oxidation of the mechanically harvested grapes crushed in the vineyard has recently been developed (15, 17).

The enzymatic oxidative activity is at first enhanced by the hyperoxygenation and then quickly diminishes. This leads to a rapid polymerization of the phenolic substances which coprecipitate with the proteins. The resulting wines have a high resistance to the 'browning reactions', similar to that of the wines produced by SO_2 addition. Some differences between these wines are also due to aroma characteristics, as confirmed by sensorial evaluation (3).

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In this paper, the effects of both the grape harvesting technique and wine fining treatments on the wine aroma are reported. The research, supported by C.N.R. (National Research Council of Italy) was carried out by a work team of the Faculty of Agriculture of the University of Bologna in a project regarding vine cultivation, harvesting techniques (4), crushed grape, must and wine treatments (16). The object of the research was in particular to establish whether wine quality is affected by mechanical harvesting of the grapes.

Material and methods

Trebbiano white grapes in normally healthy and ripening state were picked by hand or harvested by means of a vertical V.I. Emme E.S.A.V.E. (Ente Studi Assistenza Viticola Enologica) shake equipped with a Garolla-type crusher (5). 3 t of grapes were used for each trial. The technical procedures are shown in Fig. 1.

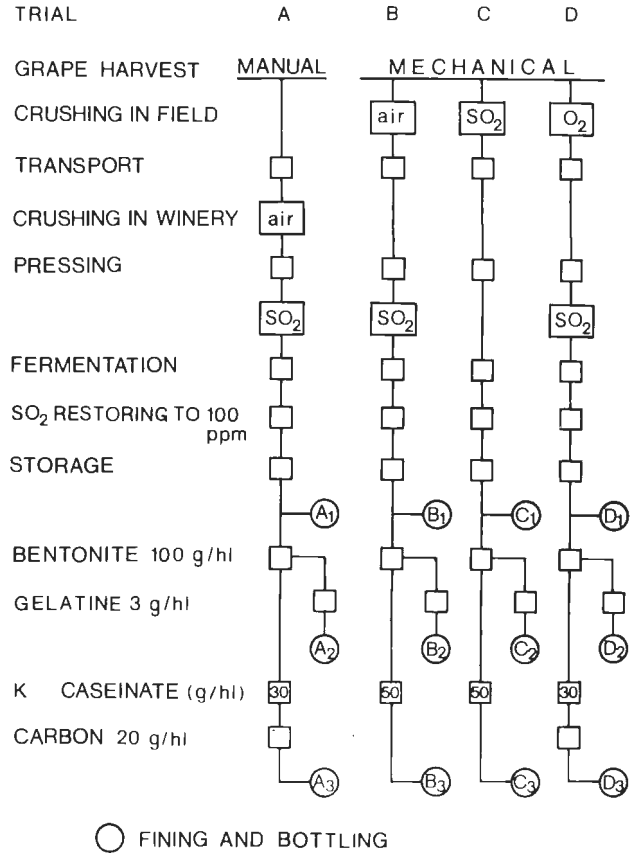


Fig. 1: Technical procedures of winemaking from Trebbiano grapes harvested by hand or by machine.

Schéma des essais de vinification de raisins Trebbiano récoltés soit à la main, soit mécaniquement.

The mechanically harvested grapes were: i) directly crushed and stemmed outside in the vineyard (trial B), ii) in an oxygen-rich atmosphere to saturate the juice (trial D), and iii) with addition of 100 ppm of SO_2 (trial C). The crushed grapes were transported by gondola trucks to the winery (Tebano Experimental Winery of the Research Center of Viticulture and Enology, University of Bologna, Ravenna, Italy) within about 3 h and the free-run juice was extracted by continuous pressing.

The hand picked grapes were crushed after their transport to the winery and pressed as above (trial A).

The 4 juices were fermented to dryness, at temperatures below 20 °C, by inoculation of pure yeast starter (*Saccharomyces cerevisiae* 404 I.M.I.A.) in presence of 100 ppm SO_2. At racking, the total SO_2 content was restored to 100 ppm.

After 6 months' storage, each wine was subdivided in 3 lots, i.e., one without treatment (series 1) and the other two differently clarified by the addition of bentonite and gelatin (letters 2) or bentonite, potassium caseinate and activated carbon (letters 3).

At bottling, each lot was filtered through diatomaceous earth after stabilization by addition of 10 g/hl of metatartaric acid and 20 g/hl of anti-oxidizer (ascorbic acid, citric acid and potassium metabisulfite).

The samples were blind-tested by 60 judges (experts and normal consumers) according to the A.E.I. (Italian Association of Enologists) method to evaluate taste.

The volatile components were extracted in a turbo-mixer at 20 °C with an azeotropic mixture of pentane : methylene chloride (7 : 3, v/v), in the ratio of 1 l of solvent/l of wine. 2 ml of a 0.5 % methyl palmitate solution were added to avoid losses in the extract concentration, which was extracted in a vacuum at 20 °C yielding a final volume to 1 ml (1).

The GLC quantitative determination was carried out by the internal standard technique using 1 ml of a 0.05 % ethyl myristate solution/l of wine.

The gas chromatographic analyses were carried out using a Carlo Erba Mod. 2900 apparatus equipped with a flame ionizing detector. A glass capillary column with 20M Carbowax, 25 m long and 0,25 mm inside diameter was used with an on-column injection system. The rate of temperature increase was programmed at 2 °C/min between 50 and 200 °C. The detector temperature was maintained at 250 °C and the hydrogen gas flow at 1 ml/min.

Identification of the individual components was carried out using combined gas chromatograph-electronic impact mass spectrometry and chemical ionization mass spectrometry with a Hewlett-Packard Mod. 5985 apparatus, operating under the same GLC conditions. The carrier gas was helium, source temperature 200 °C, electron energy 70 eV and emission current 300 μA. The scanning velocity was 450 mass units/s. Methane and isobutane were used as reactive gases for chemical ionization.

The GLC retention times and the MS fragmentation spectra were compared to those of the pure compounds specifically synthesized for the purpose.

The analysis of variance and the F statistic values were calculated by a Texas Instrument Program.

Results and discussion

The GLC histograms of the aroma profiles of the non-treated wines are reported in Fig. 2.

The harvesting techniques and the field treatments of the crushed grapes slightly influence the quantity of volatile compounds but not their quality. In fact, the GLC/MS

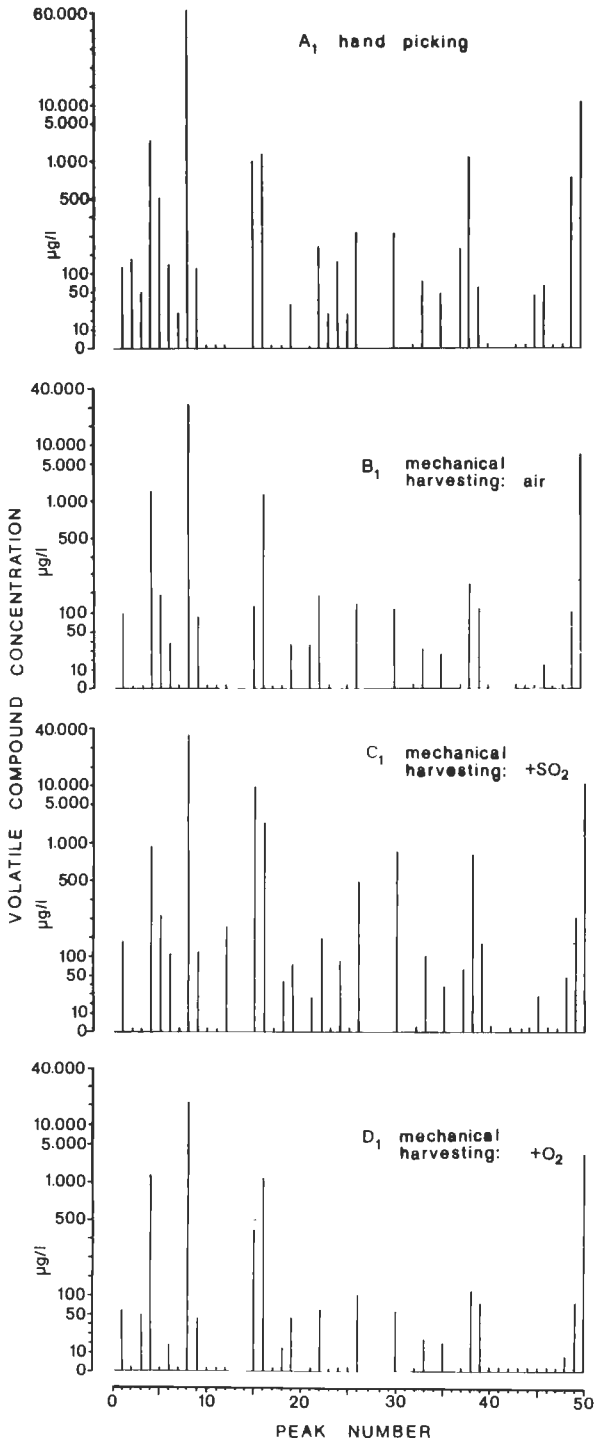
Volatile compounds identified by GLC/MS and their values of the ratio between variance with two-way criteria of classification, for the source of variability within the harvesting techniques (F_c) and within the clarification treatments (F_r) · Significant variations at 1 % level (**) and 5 % (*)

Composants volatils identifiés au moyen de CPG et CPG/SM et leurs valeurs de variance avec deux critères de classification, pour la source de variance due à la technique de vendange (F_c) et aux traitements de stabilisation (F_r) · Niveau de significativité de 1 % (**) et 5 % (*)

Peak No.	Identified compound	Molecular weight	References	Average value $\bar{x}$ (µg/l)	Grape harvesting techniques F_c	Wine clarification treatments F_r
0	Ethanol	46	8, 12, 19, 26—28	n. d.		
1	Iso-butyl acetate	116	13, 18, 19, 22, 25, 27, 28	46	3.55	36.47**
2	Ethyl butyrate	116	8, 9, 12, 13, 18, 22, 25, 26, 27	15	1.00	1.00
3	Iso- and n-propanol	60	19, 27, 28	13	2.19	1.86
4	Iso-butanol	74	9, 18—21, 23, 24, 27, 28	965	0.96	7.70*
5	Iso-amyl acetate	130	8, 9, 12, 18, 19, 22, 25, 27, 28	202	0.69	1.22
6	Amyl acetate	130	8, 9, 12, 13, 19, 22, 25, 27, 28	43	1.00	1.00
7	Butanol	74	19, 21, 23, 24, 25, 27, 28	3	1.00	1.00
8	Iso-amyl alcohol	88	7, 12, 19—21, 23, 24, 27, 28	24 250	0.63	4.00
9	Ethyl caproate	144	8, 9, 12, 18, 19, 25, 27, 28	47	2.47	22.44**
10	Amyl alcohol	88	21, 24, 25	8	0.59	1.25
11	Hexyl acetate	144	12, 19, 22, 25, 26—28	15	0.90	1.99
12	3-Hydroxy-2-butanone	88	6, 22	49	16.27**	0.87
13	Amyl butyrate	158	8, 9, 18, 22	n. d.		
14	Amyl valerate	172	9, 26	n. d.		
15	Ethyl lactate	118	12, 19, 20, 25, 27, 28	1 780	10.25**	1.76
16	1- and 2-Hexenol	102	7, 8, 18—21, 23, 24, 25, 28	1 240	0.97	1.45
17	3-Hexenol	100	21, 23, 24, 26	9	0.45	7.20*
18	3-Ethoxy-propanol	104	13	18	5.62*	0.76
19	2-Hexenol	100	21, 23, 24, 25	43	2.03	1.15
20	Butoxy-ethanol	118		n. d.		
21	Cyclo-hexanol	100	8	6	1.00	2.84
22	Ethyl caprylate	172	8, 9, 12, 13, 18, 20, 22, 24, 27, 28	98	0.84	5.12*
23	Butyl lactate	146	25	5	2.13	0.59
24	Amyl caproate	186	8, 20, 22	182	0.31	1.09
25	2,3-Butanediol monoacetate	132	20, 21, 23	9	3.18	0.27
26	2,3-Butanediol	90	8, 27	483	1.13	2.33
27	Ethyl 3-hydroxy-caproate	160	20, 24	n. d.		
28	Ethyl nonanoate	186	22, 28	n. d.		

29	Ethyl 2-hydroxy-caproate	160	6, 21, 28	n. d.		
30	Amyl lactate	160	8, 13, 25	578	0.58	0.33
31	Butyric acid	88	8, 11, 14, 28	n. d.		
32	Hexyl caproate	200	11	15	1.43	1.83
33	γ-Butyrolactone	86	13, 19, 20, 23, 24, 27, 28	46	20.12**	3.65
34	Valeric acid	102	8, 10, 13, 14, 27, 28	n. d.		
35	Ethyl caprate	200	8, 9, 13, 18—20, 22, 24, 25, 27, 28	31	0.36	0.37
36	Methyl laurate	214	19	n. d.		
37	Amyl caprylate	214	9, 18, 20, 24, 25	322	0.48	1.34
38	Diethyl succinate	174	8, 9, 12, 13, 18—20, 22, 24, 25, 27, 28	356	3.75	3.57
39	Unknown	162		56	0.14	5.42*
40	Hexyl lactate	174	8	25	2.61	2.22
41	Amyl caprylate	214	9, 18, 20, 24, 25	n. d.		
42	Dimethyl suberate	202		n. d.		
43	Butyl caprate	228	9, 18, 24	53	0.46	1.08
44	Butyl ethyl succinate	164	22, 24	9	2.42	1.12
45	2-Phenyl-ethyl acetate	164	9, 12, 13, 18—20, 22, 26, 28	123	1.75	1.78
46	Hexyl caprylate +	228	9, 19, 23	278	0.49	0.42
46	Capronic acid	116	8, 11, 13, 14, 27, 28	n. d.		
47	Ethyl laurate	228	8, 10, 13, 19, 24, 28	97	1.17	1.01
48	Benzyl alcohol	108	9, 20, 21, 23, 24, 25	72	0.75	0.91
49	Amyl caprate	242	8, 9, 24	407	0.55	2.00
50	2-Phenyl-ethyl alcohol	122	8, 12, 13, 18, 19, 21, 23, 24, 25, 27, 28	11 888	0.96	0.28
51	Diethyl malate	190	8, 9, 13, 20, 21, 24, 27, 28	n. d.		
52	Caprylic acid	140	8, 10, 13, 14, 27, 28	n. d.		
53	Ethyl hexyl succinate	230		n. d.		
54	Diamyl succinate	258	19, 24	n. d.		
55	Ethyl phenol	122	24	n. d.		
56	Ethoxy-γ-butyrolactone	130	20	n. d.		
57	Methyl 3-hydroxy-caprilate	209		n. d.		
58	Carboethoxy-γ-butyrolactone	158	20, 24	n. d.		
59	Ethyl amyl malate	232		n. d.		
60	Methyl myristate		Internal standard			
61	Capric acid	172	10, 13, 14, 27, 28	n. d.		
62	Methyl palmitate		Internal standard			

n. d. = Not determined.
*, ** = Significant at 5 % and 1 % level.


A1 hand picking
B1 mechanical harvesting: air
C1 mechanical harvesting: +SO2
D1 mechanical harvesting: +O2
VOLATILE COMPOUND CONCENTRATION
µg/l
60.000
10.000
5.000
1.000
500
100
50
10
0
40.000
0
10
20
30
40
50
PEAK NUMBER

analyses show that the same compounds are always present but vary from traces to sensible amounts depending on the sample.

Wine extract components identified by the GLC retention times and the MS fragmentation, are listed in the table.

The molecular weights reported are those determined by chemical ionization mass spectrometry. Average values for some components were not determined either because they were present only in traces or because they could not be completely resolved chromatographically.

The compounds not yet indicated in the literature are those listed as peak numbers 20 (butoxy-ethanol), 42 (dimethyl suberate), 53 (ethyl hexyl succinate), 57 (methyl 3-hydroxy-caprilate), and 59 (ethyl amyl malate).

The compound relative to peak 39 could not be identified. Chemical ionization mass spectrometry shows a molecular ion at m/e 162, whereas the spectra produced by electronic impact do not show the molecular ion but rather an intense peak at m/e 106 (base peak) with significant fragments at m/e 61 and m/e 47. However, the data are not sufficient to permit any hypotheses as to the nature of this compound, although it was found to contain sulphur.

In general, hand picking (A_1) results in a wine richer in volatile components than that obtained with mechanically harvested grapes (B_1). In addition, with mechanical harvesting, the hyperoxygenation treatment of the crushed grapes leads to a flattening of the gas chromatographic profile (D_1).

In contrast, the SO_2 addition during crushing in the vineyard increases the volatile content of the wine (C_1).

In order to better illustrate these differences, the same table also shows the values of the ratio between variances (from FISHER and YATES or from SNEDECOR) or more simply the F values obtained from the analyses of variance with two criteria of classification; F_c shows the value for the sources of variability within the harvesting techniques and F_r that within the various wine clarification treatments; $\bar{x}$ shows the average content referred to the internal standard. We listed only the components which were present in significant amounts.

First of all, for the majority of compounds present, there is no significant difference in composition between the wines produced from hand or mechanically picked grapes. In fact only 4 compounds vary significantly (at the 5 % level of error probability) as reported in Fig. 3. In order to study the influence of the harvesting technique alone, compensation for the possible influence of the clarification techniques was made by calculating the average values of the various compounds for 3 wines harvested by the same technique but clarified by different methods. In particular, mechanical harvesting increases 3-hydroxy-butanone and 3-ethoxy-propanol content, but lowers the amounts of ethyl lactate and γ-butylrolactone. In contrast, the addition of SO_2 in the crushing phase leads to an increase in these compounds, due to the higher maceration needed to preserve against alterations.

Obviously, during the transport of crushed grapes to the winery oxidation and microbiological phenomena take place which result in some changes responsible for

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Fig. 2: GLC profiles of the volatile components extracted from the non-treated wines obtained from grapes harvested by hand (A_1) or by machine (B_1) and crushed with sulphur dioxide (C_1) or with hyperoxygenation (D_1).

Histogrammes des profils gaschromatographiques des composants volatils des vins de raisins récoltés à la main (A_1) ou avec des vendangeuses mécaniques (B_1) et foulés sous atmosphère d'anhydride sulfureux (C_1) ou d'oxygène (D_1).

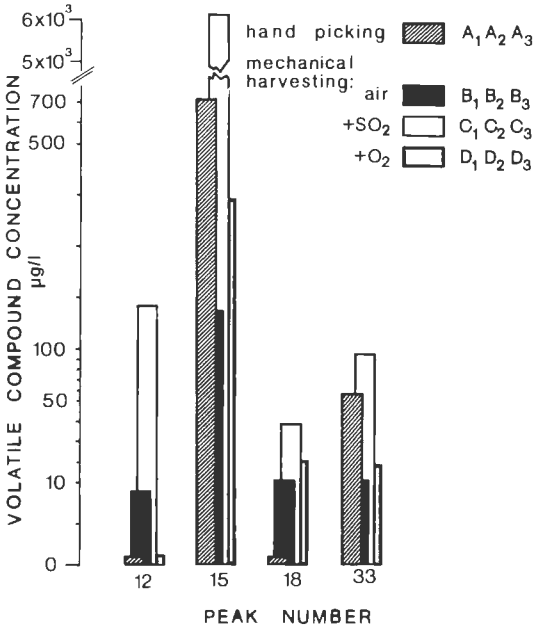

Fig. 3: Histograms of the volatile compounds which show significant variations in the wines resulting from different harvesting techniques (average value of each group of 3 wines).

Histogrammes de composants volatils qui subissent des modifications significatives en fonction des différents types de récolte (moyenne de chaque groupe de 3 vins).

the decrease in volatile compounds and for the accumulation of molecules containing a greater number of oxygen atoms.

Independently of the stabilizing effect on wine colour, the hyperoxygenation of the mechanically harvested crushed grapes accentuates these phenomena.

Generally, the stabilization treatments on the wine (Table) have an influence on compounds other than those affected by the type of harvesting.

In order to study the influence of the clarification treatments alone, compensation for the possible influence of the harvesting techniques employed was obtained as follows: Statistical evaluation was made on each lot of 4 wines, each wine clarified by the same method but harvested by a different technique. Therefore, the average values of the 4 wines for each type of treatment are indication of the effect of the clarification treatments on the chemical composition of the wine. Fig. 4 shows the histograms relative to the substances which are significantly affected (at the 5 % level of error probability) by the clarification treatments, i.e.: No. 1, isobutyl acetate; 4, isobutanol; 9, ethyl capronate; 17, 3-hexanol; 22, ethyl caprylate, and 39, a still unidentified sulfur-containing compound. With the exception of peak 17, both fining treatments cause a decrease in the amount of volatile compounds, because of their evaporation during the pump mixing in the clarification phase. In confirmation of this, the esters and the more volatile compounds showed the greatest decreases. It can be hypothesized that the stabilization treatments promote losses due to volatilization and thus, the more volatile the

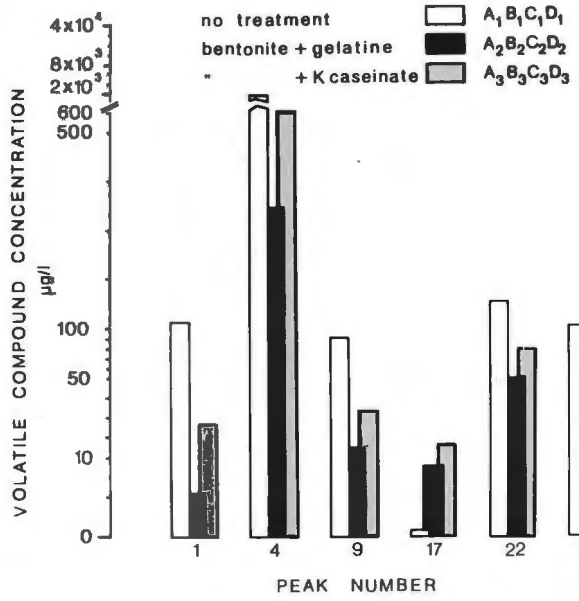

Fig. 4: Histograms of the volatile compounds which show significant variations in the wines resulting from different clarification treatments (average value of each group of 4 wines).

Histogrammes des composants volatils qui subissent des modifications significatives en fonction des différents traitements de stabilisation (moyenne de chaque groupe de 4 vins).

compound, the greater the losses. The data obtained indicate that the iso-butyl acetate is particularly affected by this phenomenon, whereas a similar effect is not seen for the analogous superior acetates, e.g. iso-amyl acetate, amyl acetate and hexyl acetate.

The bentonite and potassium caseinate treatment gives rise to wines having a relatively richer aroma than those to which only bentonite has been added. In fact, it is known that the clarification with potassium caseinate enhances white wine aroma (2), even if generally each wine treatment lowers the quality.

The taste tests by a commission of judges did not show any significant differences caused by either the type of harvesting or clarification treatments used. It should be remembered, however, that in judging the total rating, aroma represents only one of the components in examination and other parameters such as color, taste, harmony, etc. are of greater importance. Even so, the final evaluations checked on the A.E.I. test forms depend less on factors arising from the stabilizing treatments than on factors correlated with the harvesting techniques.

In particular, the wines produced from mechanically harvested grapes and treated with oxygen at crushing have a flavor which is described as 'bitter' or 'grassy', very far from the typical Trebbiano taste. The wines from SO_2 added grapes have an oxidized flavor which could be correlated to the greater content of polyphenols.

In conclusion, the results of this study show that in this white wine the harvesting techniques do not cause substantial differences in most volatile compounds. Only 4 out of more than 60 compounds identified showed statistically significant variations

mainly due to the type of treatment carried out with the crushed grapes in the vineyard, which influences the oxidation processes.

For this reason, the mechanical harvesting combined with the stemming and crushing in vineyard can replace the hand picking of white grapes. However, it should also be noted that the Trebbiano grapes we tested are poor in flavor, so their damage may have minor influence on the wine quality.

On the other hand, great care is needed in the transport of crushed grapes to the winery, shortening the time and using inert gas atmosphere. In fact, the addition of SO_2 at crushing prevents oxidative alterations but improves the extraction phenomena and lowers the quality. Also the addition of O_2 (hyperoxygenation) was not found to be suitable for this purpose as it flattens the wine aroma profiles, but may be useful in the winemaking of common wines from unhealthy grapes.

The clarification treatments carried out on these wines also had negligible effects on the majority of the compounds, causing sensible reductions in the contents of only a few of the more volatile components, that is to say, more care is needed in the wine treatments to prevent losses by volatilization.

Summary

Trebbiano white grapes were harvested both by hand and mechanically, with the addition of O_2 and SO_2 at crushing in the vineyard. After wine-making in standard conditions, the wines were clarified with different fining agents.

The wines were tested to identify the volatile components. The method developed included solvent extraction, concentration, GLC and GLC/MS analysis. Over 60 compounds were identified, some of which were previously unidentified.

Only some volatile components were significantly affected by the harvesting techniques or by clarification treatments. The hyperoxygenation of the crushed grapes lowered the aroma profile, improving the content of the more oxidized molecules. The SO_2 addition increased maceration phenomena, but decreased wine stability to browning reactions. The wine finishing with bentonite and other clarifying additives resulted in a loss of some aroma compounds due to volatilization.

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