Quantitative Changes of Some Volatile Components in Vernaccia di Oristano (a Sardinian Sherry-like Wine) during Maturation

Alberta Carnacini,[†] Andrea Antonelli,[‡] Guido C. Galletti,*[,§] Nadia Natali,[‡] and Giovanni A. Farris[||]

Dipartimento di Scienze e Tecnologie Agro Forestali e Ambientali, University of Reggio Calabria, Piazza S. Francesco 4, 89061 Gallina (RC), Italy, Istituto di Industrie Agrarie, University of Bologna, Via S. Giacomo 7, 40126 Bologna, Italy, Dipartimento di Chimica "G. Ciamician", University of Bologna, Via G. Selmi 2, 40126 Bologna, Italy, and Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agroalimentari, University of Sassari, Viale Italia 39, 07100 Sassari, Italy

Vernaccia di Oristano, a sherry-like wine produced in Sardinia, Italy, was subjected to biological aging after inoculation with *Saccharomyces cerevisiae* var. bayanus (strain 1043) and *S. cerevisiae* var. prostoserdovii (strain 1739) and to aging under sterile conditions. Samples were withdrawn at three different stages of maturation of the sherry-like wine, and the volatiles were analyzed by gas chromatography and gas chromatography/mass spectrometry. Forty compounds were identified comprising alcohols, acids, esters, and dioxolanes. Analysis of variance showed significant effects of both aging conditions and duration on most of the volatiles, with differences maximized at the final sampling time, i.e., 20 days after film formation.

Keywords: *Vernaccia di Oristano; sherry; flavor; gas chromatography/mass spectrometry; Saccharomyces cerevisiae var. bayanus; Saccharomyces cerevisiae var. prostoserdovii; yeasts*

INTRODUCTION

The volatile composition characterizes sherry wine quality and its production technologies (Webb and Noble, 1976; Criddle *et al.*, 1983; Brock *et al.*, 1984; Nykanen, 1986). A previous experiment has dealt with the identification and quantification of volatile components in Vernaccia di Oristano (an Italian sherry-like wine produced in Sardinia since the 11th century) under different aging conditions, i.e., sterile conditions and biological aging after inoculation with *Saccharomyces cerevisiae* var. *bayanus* (strain 1043) (SCB) and *S. cerevisiae* var. *prostoserdovii* (strain 1739) (SCP) (Galletti *et al.*, 1996). The latter strain resulted in a product characterized by a more intense and equilibrated scent, as suggested by other authors (Fatichenti *et al.*, 1983). However, it would be difficult to prefer one strain rather than the other because of the different behavior of the two strains (Galletti *et al.*, 1996). Such preliminary observations prompted us to monitor the volatile components in Vernaccia di Oristano at various intervals over the entire period of maturation, checking the effects of different aging conditions and durations, in the attempt to optimize the production technology of such a sherry-like wine.

EXPERIMENTAL PROCEDURES

Vernaccia di Oristano (100 L), 6 months after 1993 vintage in its typical area, was aged under sterile conditions and by addition of SCB and SCP. Flavor components were analyzed by gas chromatography in the original wine and in samples collected at three different times of aging, namely, at the formation of the flor film (8 and 18 days after inoculation for SCB and SCP, respectively) (T1), 10 days after film formation (T2), and 20 days after film formation (T3). Aging and analytical conditions were described elsewhere (Galletti *et al.*, 1996). Briefly, Vernaccia di Oristano was aged in nine 10-L flasks. Three flasks were kept as controls under sterile conditions, three flasks were inoculated with SCB, and the remaining three flasks were inoculated with SCP. All procedures were repeated twice.

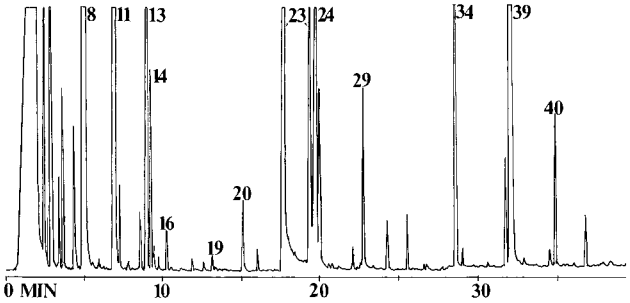

Figure 1. Gas chromatographic profile of the aromatic components of a typical Vernaccia di Oristano sample. The main peaks are numbered as in Table 1. Number 23 refers to both optical isomers of 2,3-dihydroxybutane.

Acetaldehyde, acetone, ethyl acetate, and alcohols (methanol, propanol, 2-methylpropanol, and 3- and 2-methylbutanol) were determined by gas chromatography (GC) after distillation (AOAC, 1984) [GC conditions: Hewlett Packard model 5710 A gas chromatograph (Hewlett Packard, Palo Alto, CA), column Carbowax 1500 (230 g kg^{-1} on Chromosorb W-AW, 2 m × 3 mm i.d.), oven at 70 °C, nitrogen carrier flow of 30 mL min^{-1}, injector and flame ionization detector at 250 °C]. Ethanol was determined by measuring the density of the diluted distillate. Flavor components were determined by GC and gas chromatography/mass spectrometry (GC/MS) after extraction and concentration of the volatiles by means of a column packed with Extrelut resin (Bracco Merck, Milan, Italy) (Gerbi *et al.*, 1992; Hill and Ferris, 1927) [GC conditions: Carlo Erba model GC 6000 gas chromatograph, column Megawax (25 m × 0.25 mm i.d., 0.25 μm film thickness) operated from 40 to 60 to 250 °C at, respectively, 20 and 2.5 °C/min, holding the initial and intermediate temperature for

* To whom correspondence should be addressed (telephone +39 51 259533; fax +39 51 259456; e-mail galletti@ciam.unibo.it).
[†] University of Reggio Calabria.
[‡] Istituto di Industrie Agrarie, University of Bologna.
[§] Dipartimento di Chimica, University of Bologna.
[||] University of Sassari.

S0021-8561(96)00653-X CCC: $14.00

Table 1. Volatile Composition of Vernaccia di Oristano under Sterile (Control, C) and Biological Conditions (*S. cerevisiae* Var. *bayanus*, SCB, and *S. cerevisiae* Var. *prostoserdovii*, SCP) at Three Different Aging Times[a]

		T1			T2			T3					
compound	O	C	SCB	SCP	C	SCB	SCP	C	SCB	SCP	A	B	A × B
1. ethanol	13.6	12.3a	12.7b	12.8c	11.3a	11.7b	11.0c	9.9b	9.9b	9.4a	**	**	**
2. acetaldehyde	4.4×10^2	1.5×10^2a	2.2×10^2b	2.2×10^2b	82a	6.1×10^2c	4.3×10^2b	47a	4.5×10^2c	3.2×10^2b	**	**	**
3. acetone	2	18b	3a	3a	15b	2a	3a	10b	3a	8ab	**	ns	**
4. ethyl acetate	42	22	20	14	11a	37c	19b	5a	18b	8a	**	**	**
5. methanol	42	69a	80b	67a	63b	60b	46a	52b	55b	31a	*	**	**
6. propanol	24	26	27	25	26a	47b	26a	19a	43b	19a	**	ns	**
7. 2-methylpropanol	42	40	42	36	38a	52b	34a	31a	77c	40b	**	*	**
8. 3- and 2-methylbutanol	3.3×10^2	3.1×10^2b	3.2×10^2b	2.8×10^2a	2.9×10^2b	2.9×10^2b	2.4×10^2a	2.3×10^2b	2.9×10^2c	2.1×10^2a	**	**	**
9. pentanol	1.0×10^{-1}	1.2×10^{-1}b	7.5×10^{-2}a	5.6×10^{-2}a	1.5×10^{-1}	1.0×10^{-1}	1.4×10^{-1}	7.3×10^{-2}	7.8×10^{-2}	1.3×10^{-1}	**	**	**
10. hexyl acetate	3.7×10^{-2}	4.1×10^{-2}a	4.6×10^{-2}a	1.0×10^{-1}b	9.3×10^{-2}a	1.1×10^{-1}a	3.2×10^{-1}b	2.7×10^{-1}b	3.6×10^{-2}a	4.0×10^{-2}a	**	**	**
11. 3-hydroxy-2-butanone	6.5	6.9a	14b	18b	6.9a	1.5×10^2b	1.7×10^2c	5.4a	1.7×10^2b	2.2×10^2c	**	**	**
12. 3-methylpentanol	8.6×10^{-2}	9.7×10^{-2}b	7.9×10^{-2}b	5.9×10^{-2}a	9.3×10^{-2}	1.0×10^{-1}	8.9×10^{-2}	6.8×10^{-2}a	4.2×10^{-2}a	1.1×10^{-1}b	**	**	**
13. ethyl lactate	84	83	84	71	90	74	89	91c	46a	63b	**	**	**
14. hexanol	1.4	1.3	1.2	9.6×10^{-1}	1.2	1.1	1.1	9.9×10^{-1}	8.4×10^{-1}	8.4×10^{-1}	*	**	ns
15. *trans*-3-hexen-1-ol	2.9×10^{-2}	3.4×10^{-2}	3.2×10^{-2}	2.2×10^{-2}	3.5×10^{-2}a	9.5×10^{-2}b	1.0×10^{-1}b	3.1×10^{-2}a	3.3×10^{-1}b	4.4×10^{-1}c	**	**	**
16. 3-ethoxy-1-propanol	9.0×10^{-1}	1.0	1.2	9.1×10^{-1}	9.4×10^{-1}a	2.8c	1.8b	8.2×10^{-1}a	1.8b	2.0b	**	**	**
17. *cis*-3-hexen-1-ol	1.0×10^{-1}	1.2×10^{-1}ab	1.3×10^{-1}b	8.3×10^{-2}a	1.1×10^{-1}	1.2×10^{-1}	1.3×10^{-1}	9.0×10^{-2}a	9.1×10^{-2}a	1.7×10^{-1}b	*	**	**
18. ethyl octanoate	1.8×10^{-1}	3.5×10^{-2}	8.5×10^{-2}	8.0×10^{-2}	1.1×10^{-1}b	5.3×10^{-2}a	1.1×10^{-1}b	7.1×10^{-2}b	3.5×10^{-2}a	6.5×10^{-2}b	**	ns	**
19. acetic acid	1.7	2.3b	1.7b	4.0×10^{-1}a	6.8×10^{-1}ab	7.7×10^{-1}b	5.8×10^{-1}a	5.9×10^{-1}b	2.9×10^{-1}a	4.9×10^{-1}ab	**	**	**
20. *cis*-5-hydroxy-2-methyl-1,3-dioxane	2.4	2.1	1.8	2.4	1.9a	4.1b	5.1b	1.7a	2.6ab	3.9b	**	**	**
21. ethyl 3-hydroxybutanoate	6.1×10^{-1}	6.2×10^{-1}	6.0×10^{-1}	5.3×10^{-1}	6.3×10^{-1}	7.6×10^{-1}	9.7×10^{-1}	5.8×10^{-1}a	6.9×10^{-1}a	1.1b	**	**	**
22. propanoic acid	8.1×10^{-1}	1.1b	1.1b	2.1×10^{-1}a	3.0×10^{-1}b	6.6×10^{-1}c	5.4×10^{-2}a	3.5×10^{-1}b	4.9×10^{-2}a	6.1×10^{-2}a	**	**	**
23. 2,3-dihydroxybutane	95	1.1×10^2b	1.1×10^2b	92a	95	95	90	73b	53a	95c	**	**	**
24. 4-butanolide	29	30b	32c	28a	35b	31a	39c	32b	25a	37a	**	**	**
25. *cis*-4-hydroxy-2-methyl-1,3-dioxolane	5.4	4.6	5.4	4.9	3.6a	6.4b	7.2c	2.9a	4.8b	6.2b	**	**	**
26. butanoic acid	1.2	2.2c	1.5b	5.3×10^{-1}a	1.2b	1.3b	1.6×10^{-1}a	5.1×10^{-1}c	6.3×10^{-2}a	2.7×10^{-1}b	**	**	**
27. ethyl decanoate	9.9×10^{-2}	4.7×10^{-2}ab	5.9×10^{-2}b	3.2×10^{-2}a	5.0×10^{-2}a	2.2×10^{-1}b	9.5×10^{-2}ab	1.2×10^{-2}a	1.3×10^{-2}ab	1.4×10^{-2}c	**	**	**
28. *trans*-4-hydroxy-2-methyl-1,3-dioxolane	1.4	2.0b	1.9b	7.8×10^{-1}a	8.9×10^{-1}b	1.5c	3.6×10^{-2}a	8.0×10^{-2}b	2.5×10^{-2}a	3.0×10^{-2}a	**	**	**
29. diethyl butanedioate	10	9.8	8.2	8.6	9.8	9.8	11	8.6	7.2	11	**	**	**
30. 3-(methylthio)-1-propanol	2.5	2.7c	2.3b	1.9a	2.2b	1.8a	1.7a	1.8c	1.4a	1.6b	**	**	**
31. pentanoic acid	9.9×10^{-2}	1.1×10^{-1}b	2.0×10^{-1}c	4.5×10^{-2}a	1.5×10^{-1}b	1.4×10^{-1}b	5.4×10^{-2}a	8.0×10^{-2}	5.0×10^{-2}	6.8×10^{-2}	**	**	**
32. 1,3-dihydroxypropyl monoacetate	2.7	3.3b	3.2b	2.4a	2.9	2.9	2.7	2.5c	1.5a	1.8b	**	**	**
33. phenethyl acetate	5.4×10^{-2}	7.0×10^{-2}a	8.7×10^{-2}b	6.6×10^{-2}a	6.8×10^{-2}a	1.2×10^{-1}b	1.4×10^{-1}c	6.3×10^{-2}	8.6×10^{-2}	1.3×10^{-2}	**	**	**
34. ethyl 4-hydroxybutanoate	19	20b	20b	16a	18b	23b	23b	16a	26b	25b	**	**	**
35. *trans*-5-hydroxy-2-methyl-1,3-dioxane	5.0×10^{-1}	6.5×10^{-1}b	6.2×10^{-1}b	4.8×10^{-1}a	4.7×10^{-1}a	9.2×10^{-1}c	6.6×10^{-1}b	3.5×10^{-1}a	3.7×10^{-1}a	6.8×10^{-1}b	**	**	**
36. ethyl dodecanoate	6.6×10^{-2}	2.0×10^{-2}a	4.7×10^{-2}b	2.1×10^{-2}a	3.9×10^{-2}	2.7×10^{-2}	3.2×10^{-2}	2.8×10^{-2}	2.9×10^{-2}	5.1×10^{-2}	ns	*	**
37. hexanoic acid	4.3×10^{-1}	4.5×10^{-1}b	5.7×10^{-1}c	2.6×10^{-1}a	3.1×10^{-1}b	3.6×10^{-1}b	2.1×10^{-2}a	2.9×10^{-1}b	1.9×10^{-2}a	9.7×10^{-2}a	**	**	**
38. benzyl alcohol	1.1×10^{-1}	1.1×10^{-1}	1.4×10^{-1}	1.1×10^{-1}	1.2×10^{-1}	1.4×10^{-1}	1.8×10^{-1}	1.2×10^{-1}	1.5×10^{-1}	1.9×10^{-1}	**	**	**
39. 2-phenylethanol	1.6×10^2	1.6×10^2	1.5×10^2	1.4×10^2	1.7×10^2	1.8×10^2	1.8×10^2	1.7×10^2a	2.0×10^2b	2.5×10^2c	**	**	**
40. octanoic acid	7.5	6.5b	8.1b	3.1a	4.5c	3.8b	2.0a	3.5b	3.1×10^{-1}a	3.4×10^{-1}a	**	**	**

[a] T1, formation of the film; T2, 10 days later; and T3, 20 days later. The composition of the original wine (O) is also reported. Figures are expressed in mg/L and ethanol is expressed as percentage (v/v). Numbers with different letters differ at the $P < 0.01$ level (Tukey's test), for each compound and within each maturation time; A, aging conditions (C, SCB, and SCP); B, maturation times (T1, T2, and T3); A × B, interaction A × B; ns, non-significant differences; **, differences significant at the $P < 0.01$ level; *, differences significant at the $P < 0.05$ level.

30 s and 2 min, respectively, hydrogen carrier flow of 3 mL min^{-1}, injector (split closed for 30 s) and flame ionization detector at 250 °C]. GC/MS analyses were carried out with a Fisons QMD 1000 (Fisons, Milan, Italy) instrument, maintaining the GC conditions described above and recording mass spectra from 30 to 400 m/z at 70 and 20 eV. Quantitative analysis of the flavor components was carried out by means of an internal standard (*n*-dodecanol) added to the samples prior to concentration with Extrelut resins. Extraction yields exceeded 80% for all compounds (Gerbi *et al.*, 1992).

A 3 × 3 factorial analysis of variance (ANOVA) was used for each compound quantified by GC and for ethanol: three aging conditions (sterile or control, SCB, and SCP inoculations) and three aging times (T1, T2, T3). Tukey's test (Miller and Miller, 1993) was used to determine the least significant differences between the three aging conditions for the various compounds at each aging time. StatGraphics package 4.0 (Manugistic Inc., Rockville, MD) was used for statistic calculations.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram obtained from a Vernaccia di Oristano sample. Table 1 shows quantitative results and ANOVA for ethanol and flavor components. Almost all compounds showed an aging condition effect at the $P < 0.01$ level, with the exceptions of ethyl dodecanoate, which was not significantly affected by the aging condition, and of methanol, hexanol, and *cis*-3-hexen-1-ol, which showed effects at the $P < 0.05$ level. Likewise, aging duration affected almost all compounds at the $P < 0.01$ level, with the exceptions of acetone, propanol, and *cis*-3-hexen-1-ol, which showed nonsignificant differences, and of 2-methylpropanol and ethyl dodecanoate, which were affected at the $P < 0.05$ level.

As to the starting wine, a high concentration of acetaldehyde suggests that some fermentation processes were active probably as a consequence of the poorly controlled production technology, which is typical of the small Sardinian industries (Farris *et al.*, 1978; Fatichenti *et al.*, 1979). The decrease of ethanol percentage during maturation was statistically different depending on aging conditions and probably due not only to evaporation but also to the microbial activity of the flor yeasts. These oxidize ethanol and use it for respiration and for the synthesis of lipids within the yeast cell (Martinez de la Ossa *et al.*, 1987). In general, the quantities of most of the flavor components increased as a consequence of the treatment with yeasts, and particularly by treatment with SCP. Among the few components with an opposite trend, of particular interest is the decrease of all carboxylic acids.

Most of the compounds showed a significant yeast strain effect at $P < 0.01$ level at various aging stages. Only two compounds, namely, hexanol and diethyl butanedioate, were not affected by the strain used at any sampling time. A decrease of hexanol of about 30−40% with respect to the starting wine was observed in the aged products, possibly as a result of both oxygen and yeast actions. The progress in aging tended to enhance the differences in the flavor components induced by the yeast treatment, because the number of components showing no yeast effect diminished from 14 in the first set of samples (T1) to 12 in the second (T2) and 6 in the final set (T3). This is not surprising since the whole process is a combination of oxidative phenomena and evaporation losses observed in the sterile samples and in the bio-oxidations typical of the wine inoculated with the flor yeasts.

In spite of identical experimental conditions, SCB filmed the samples 10 days earlier than SCP (see Experimental Procedures). SCP might have used some of the flavor components for its metabolism. Hence, this strain was characterized by a significantly lower content of most of the flavor components at T1 compared to the control sample under sterile conditions, and by a significantly higher content of 3-hydroxy-2-butanone, a compound typical of all bio-oxidation reactions (i.e., acetic fermentation), at the final aging time (Asai, 1968; Gerbi *et al.*, 1995). By contrast, SCB was characterized by an oxidative metabolism, as the comparatively larger number of flavor components showing nonsignificant differences with the control sample at least in the first aging time seems to suggest.

The differences between samples are more evident 10 days after film formation (T2). It is apparent that the yeasts showed the strain characters after an initial time lag. In particular, SCB produced propanol, 2-methylpropanol, and 3- and 2-methylbutanol (isomer mixture) whereas SCP metabolized them. Production and consumption of such compounds could be concomitant phenomena during the oxidative metabolism for yeasts. The particular need for nitrogen by SCB might result in a net production of higher alcohols (Amerine and Ough, 1980). SCB was also characterized by a lower content of flavor components and particularly of the ethyl esters of lactic, octanoic, and dodecanoic acids, which are of particular interest for the organoleptic properties of the aged wine.

The samples were even more differentiated at the final aging time, when only six flavor components did not show any significant effect of the aging conditions. It is interesting to note the trend of the alcohols with six carbon atoms, which originate from acids with 18 carbon atoms in grape skins (Schreier, 1979). In the sterile samples, the contents of hexanol and *cis*-3-hexen-1-ol decreased with aging whereas that of *trans*-3-hexen-1-ol remained practically constant. In contrast, by treatment with yeasts, the content of *trans*-3-hexen-1-ol increased of up to 1 order of magnitude in the final set of samples.

In conclusion, SCB and SCP strains behaved individually in leading to the development of particular quantitative distributions of the flavor components in Vernaccia di Oristano sherry-like wines. Such flavor compositions were different from that present in the sample aged under sterile conditions, which, in turn, was similar to that of the original wine. Differences were maximized 20 days after the formation of the film. Producers of Vernaccia di Oristano sherry-like wines might therefore be advised to abandon traditional practices based on aging of the wine as such and to address their efforts toward technologies based on biological aging in order to maximize the scent of the finished product.

LITERATURE CITED

AOAC. *Alcohols (Higher) and Ethyl Acetate in Distilled Liquors (9.091)*; AOAC: Washington, DC, 1984; p 184.

Amerine, M. A.; Ough, C. S. *Methods of Analysis of Must and Wines*; John Wiley and Sons: New York, 1980; pp 103−106.

Asai, T. *Acetic Acid Bacteria*; University of Tokyo Press: Tokyo, Japan, 1968; p 343.

Brock, M. L.; Kepner, R. E.; Webb, D. A. Comparison of volatiles in a Palomino wine and a submerged culture flor sherry. *Am. J. Enol. Vitic.* **1984**, *35*, 151−155.

Criddle, W. J.; Goswell, R. W.; Williams, M. A. The chemistry of sherry maturation. II. An investigation of the volatile components present in "standard" sherry base wine. *Am. J. Enol. Vitic.* **1983**, *34*, 61−71.

Farris, G. A.; Fatichenti, F.; Deiana, P.; Ceccarelli, S. L'influenza della temperatura sull'accumulo di acetaldeide da parte di *Saccharomyces prostoserdovii* in fase flor. *Riv. Vitic. Enol.* **1978**, *31*, 129−142.

Fatichenti, F.; Farris, G. A.; Deiana, P. Improved production of a Spanish-type sherry by using selected indigenous film-forming yeasts as starters. *Am. J. Enol. Vitic.* **1983**, *34*, 216−220.

Galletti, G. C.; Carnacini, A.; Antonelli, A.; Farris, G. A. Volatile composition of Vernaccia di Oristano sherry-like wine as affected by biological ageing. *J. Sci. Food Agric.* **1996**, *70*, 44−50.

Gerbi, V.; Zeppa, G.; Carnacini, A. Rapid extraction of volatile compounds in wine and vinegar using extrelut resin. *Ital. J. Food Sci.* **1992**, *4*, 259−268.

Gerbi, V.; Zeppa, G.; Antonelli, A.; Natali, N.; Carnacini, A. Evoluzione dei costituenti principali del vino e del sidro nel corso dell'acetificazione. *Ind. Bevande* **1995**, *24*, 241−246.

Hill, J. B.; Ferris, S. W. Laboratory fractionating columns. *Ind. Eng. Chem.* **1927**, *19*, 379−382.

Martinez de la Ossa, E.; Perez, L.; Caro, I. Variations of the major volatiles through ageing of sherry. *Am. J. Enol. Vitic.* **1987**, *38*, 293−297.

Miller, J. C.; Miller, J. N. *Statistics for Analytical Chemistry*; Ellis Horwood: New York, 1993; pp 153−154.

Nykanen, L. Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *Am. J. Enol. Vitic.* **1986**, *37*, 84−96.

Schreier, P. Flavor composition of wine: A review. *Crit. Rev. Food Sci. Nutr.* **1979**, *12*, 59−111.

Webb, A. D.; Noble, A. C. Aroma of sherry wines. *Biotechnol. Bioeng.* **1976**, *18*, 939−952.

Received for review August 30, 1996. Revised manuscript received March 3, 1997. Accepted March 4, 1997.[⊗]

JF960653S

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1997.