

Changes in the Composition of Alcohols and Aldehydes of C₆ Chain Length during the Alcoholic Fermentation of Grape Must

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The evolution of certain alcohols and aldehydes of C₆ chain length was investigated during alcoholic fermentation with *Saccharomyces cerevisiae* of a grape must of the white Albillo cultivar, in the presence or in the absence of sulfur dioxide. Different experimental models were also carried out in order to establish certain metabolic changes occurring during the course of the wine making. Gas chromatographic analysis was accomplished by using a 10 m × 0.85 mm (i.d.) micropacked column of Igepal CO 880 + Carbowax 20 M (80:20) (4% on desilanzed Volaspher A-2), which allows the satisfactory resolution of several C₆ alcohol and aldehyde isomers.

Alcohols and aldehydes of C₆ chain length are quite common components of several fruits and vegetables, and it is known that these compounds are originated enzymatically from linoleic and linolenic acids by aerobic oxidation (Tressl and Drawert, 1973; Stone et al., 1975; Galliard and Matthew, 1977; Cayrel et al., 1983; Hatanaka et al., 1986). Their occurrence in wines has been studied since it is related to the so-called "leafy grassy", herbaceous odor initially attributed to leaves included with mechanical harvesting (Joslin and Ough, 1978; Ramey et al., 1986). Different research groups have identified several C₆ compounds in grape and must, namely hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, (*Z*)-2-hexen-1-ol, (*E*)-3-hexen-1-ol, and 1-hexanol (Schreier et al., 1976; Schreier, 1979; Berta, 1986).

Levels of six-carbon alcohols and aldehydes in grape must depend upon several factors: the grape variety and the ripeness rate of grapes (Cordonnier and Bayonove, 1981; Martín-Alvarez et al., 1987), treatment of the must (Mesias et al., 1983), and time and temperature affecting the contact with skins (Ramey et al., 1986). Joslin and Ough (1978) have already investigated the changes occurring in the concentration of these compounds at the early phase of the alcoholic fermentation. However, the evolution of alcohols and aldehydes of C₆ chain length all throughout the fermentation of must grape and the influence of the yeast upon the changes observed in their concentration have not been sufficiently studied. The effect of elimination of SO₂ regarding these compounds has not been considered up until now in depth.

In order to show eventual variations of the amounts of the above-mentioned isomers in the medium during the course of wine making, reliable, sensitive, and very efficient analytical methods are needed. Consequently, the high performance provided by capillary columns is often required although some aspects concerning discrimination produced by inadequate sampling procedure should be carefully optimized since they could seriously affect the accuracy of the analytical determinations (Schomburg et al., 1977, 1985; Loyola et al., 1987; Reglero et al., 1988).

Micropacked columns have proven their advantages in quantitative analysis of mixtures of medium complexity as a result of their high specific efficiencies and high sample capacities (Reglero et al., 1985; Herraiz et al., 1988).

Moreover, their use for specific applications has been previously considered as an adequate alternative to capillary columns since a suitable optimization of the chromatographic selectivity is easily achievable. In this regard, analysis of volatile components of alcoholic beverages by using a packed column loaded with a mixture of Carbowax 20 M and Igepal CO 880 (20:80) has been already reported by Cabezudo et al. (1983).

On the other hand, liquid-liquid extraction with trichlorofluoromethane (Freon 11) has already shown its usefulness for obtaining a representative image of the characteristic aroma of complex mixtures such as alcoholic beverages (Hardy, 1969; Rapp et al., 1976).

The goal of this work was the study of the changes occurring in the concentrations of alcohols and aldehydes of C₆ chain length during alcoholic fermentation, in the presence or in the absence of SO₂. To this aim, grape must or synthetic media and *Saccharomyces cerevisiae* as a fermentative agent were used.

MATERIALS AND METHODS

Sterilized must from the white Albillo cultivar was fermented with *S. cerevisiae* (strain 87) provided by the Instituto de Fermentaciones Industriales, CSIC, Spain.

A culture of the above-mentioned yeast incubated at 21 °C for 48 h in a small volume of sterile must was used as inoculum (2%, v/v) for a larger volume of must (2 L).

Fermentations were carried out simultaneously in two different series, one with SO₂ (120 mg/L, added as NaHSO₃) and the other without. Evolution of C₆ volatiles during the alcoholic fermentation was established by analyzing the initial must as well as those samples taken out 2, 3, 6, 12, and 38 days after the beginning of the fermentation.

On the other hand, 1-hexanol, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, (*E*)-3-hexen-1-ol, (*Z*)-2-hexen-1-ol, (*E*)-2-hexenal, and hexanal (from Aldrich Chemie) were added, separately, at three different levels of concentration (see Table I) to aliquots (160 mL) of a sterilized medium (90 g/L of D-glucose and 4.5 g/L of yeast nitrogen base (Difco)). Each 160-mL portion was inoculated with a 48-h culture (2%, v/v) of *S. cerevisiae*, strain 87, and finally the fermentations were carried out until completion.

Prior to the chromatographic analysis itself, 250 mL of the sample was continuously extracted for 24 h with 150 mL of freshly bidistilled Freon-11. All the extractions were carried out starting from an initial medium with pH > 5. A 7-μL volume of methyl caprylate (2%, v/v, in ethanol) was added as internal stan-

Table I. Composition of Samples Obtained (mg/L) by Separate Fermentations with *S. cerevisiae* of Aliquots of a Synthetic Medium after Having Added 1-Hexanol, (Z)-3-Hexen-1-ol, (E)-2-Hexen-1-ol, (E)-3-Hexen-1-ol, (Z)-2-Hexen-1-ol, Hexanal, and (E)-2-Hexenal at Three Different Concentrations

	1-hexanol		(Z)-3-hexen-1-ol		(E)-2-hexen-1-ol		(E)-3-hexen-1-ol		(Z)-2-hexen-1-ol		hexanal		(E)-2-hexenal		control									
	19.3 ^a	9.1	4.7	4.7	21.2	10.6	4.8	21.2	10.6	4.7	23.3	11.7	5.8	24.2		12.1	6.0	20.7	10.4	5.2	21.0	10.5	5.2	
1-hexanol	14.8	6.2	4.2	-	9.9	5.3	2.4	-	1.6	-	4.0	2.4	2.2	4.2	2.5	1.5	6.7	3.7	1.9	-	-	-	-	
(Z)-3-hexen-1-ol	- ^b	-	-	18.3	8.2	4.9	-	0.1	1.8	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	
(E)-2-hexen-1-ol	-	-	-	-	0.1	0.1	-	-	20.5	9.9	5.9	-	-	0.1	0.1	0.2	-	-	-	-	-	-	-	
(E)-3-hexen-1-ol	-	-	-	0.8	0.5	0.4	-	-	-	-	-	-	-	10.3	6.3	2.7	-	-	-	-	-	-	-	
(Z)-2-hexen-1-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
hexanal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	0.1	0.1	-	-	-	-	
(E)-2-hexenal	0.4	0.2	0.2	0.2	0.1	0.2	0.1	-	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.3	0.2	0.3	0.2	0.2	0.1	
ethyl caproate	5.1	4.4	2.3	0.6	1.4	1.6	1.6	4.3	2.6	5.2	0.9	1.2	0.6	1.2	1.3	1.1	6.4	3.8	3.9	3.5	3.8	3.0	0.4	
caproic acid	0.4	0.1	0.1	-	-	-	-	0.1	0.1	0.1	-	-	-	-	-	-	0.1	0.1	0.1	0.2	0.1	0.1	-	
hexyl acetate	76.7	68.1	89.4	0.0	0.0	0.0	0.0	46.7	50.0	51.1	0.0	0.0	0.0	16.5	19.8	36.7	20.2	24.0	28.8	31.9	35.2	36.5	0.0	
recovered																								
1-hexanol (%)																								

^a Added concentration (mg/L). ^b Trace.

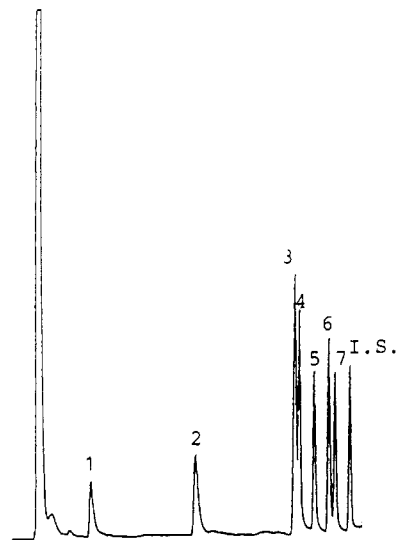


Figure 1. Chromatogram obtained with a synthetic mixture of several alcohol and aldehyde isomers of C₆ chain length. Conditions: column, 10 m × 0.85 mm (i.d.) deactivated glass Pyrex coated with Igepal CO 880 + Carbowax 20 M (80:20) (4%, w/w, on desilanzated Volaspher A-2, 120–140 mesh); carrier gas, helium; temperature program, 80–180 °C (1.5 °C/min). Peak identification: 1, hexanal; 2, (E)-2-hexenal; 3, 1-hexanol; 4, (E)-3-hexen-1-ol; 5, (Z)-3-hexen-1-ol; 6, (E)-2-hexen-1-ol; 7, (Z)-2-hexen-1-ol; IS, methyl caprylate. (See text for further details.)

standard. A 30-mL sample of the extract was then concentrated to about 0.3 mL on a Vigreux column maintained at 32 °C. Finally 5 μL of the resulting concentrate was injected into the gas chromatograph.

Analytical Procedure. The gas chromatographic analysis was performed on a Shimadzu GC-R1A gas chromatograph linked to a Shimadzu RP1 C1 integrator and equipped with a standard injector (250 °C) and a FID operated at 250 °C.

A 10 m × 0.85 mm (i.d.) micropacked column made from deactivated Pyrex tubing loaded with Igepal CO 880 + Carbowax 20 M (80:20) on desilanzated Volaspher A-2 (120–140 mesh, Merck, 4%, w/w) was used. The column was prepared according to a previously described procedure (Reglero et al., 1985). The oven was maintained at 80 °C upon injection, and it was subsequently increased at 1.5 °C/min to 180 °C. In all cases, hydrogen was used as a carrier gas (11.5 kg/cm²).

RESULTS AND DISCUSSION

Figure 1 shows the resolution achieved by using the micropacked column proposed in Material and Methods. It is interesting to point out that analysis of several isomers of alcohols and aldehydes of C₆ chain length is possible in an only run.

Chromatograms obtained from Freon extracts of the initial must and the final wine are compared in Figure 2. As can be seen, 1-hexanol and (E)-2-hexen-1-ol were found to be among the major volatile components of the must whereas smaller amounts of (Z)-3-hexen-1-ol were detected. On the other hand, it is evident that the content of 1-hexanol increases in the final wine with regard to the concentration quantified in the initial must while the level of (Z)-3-hexen-1-ol is not significantly altered and (E)-2-hexen-1-ol was only detected in the initial must.

Changes observed in the concentration of the mentioned compounds all throughout the alcoholic fermentation are given in Figure 3. It is clear the rapid reduction in (E)-2-hexen-1-ol during the early phase of the fermentation and the concurrent formation of 1-hexanol show the highest level about the second day after inoculation. After the third day, there is a slight decrease in the quantity of the last mentioned alcohol and this trend is maintained even after the end of the fermentation (at day

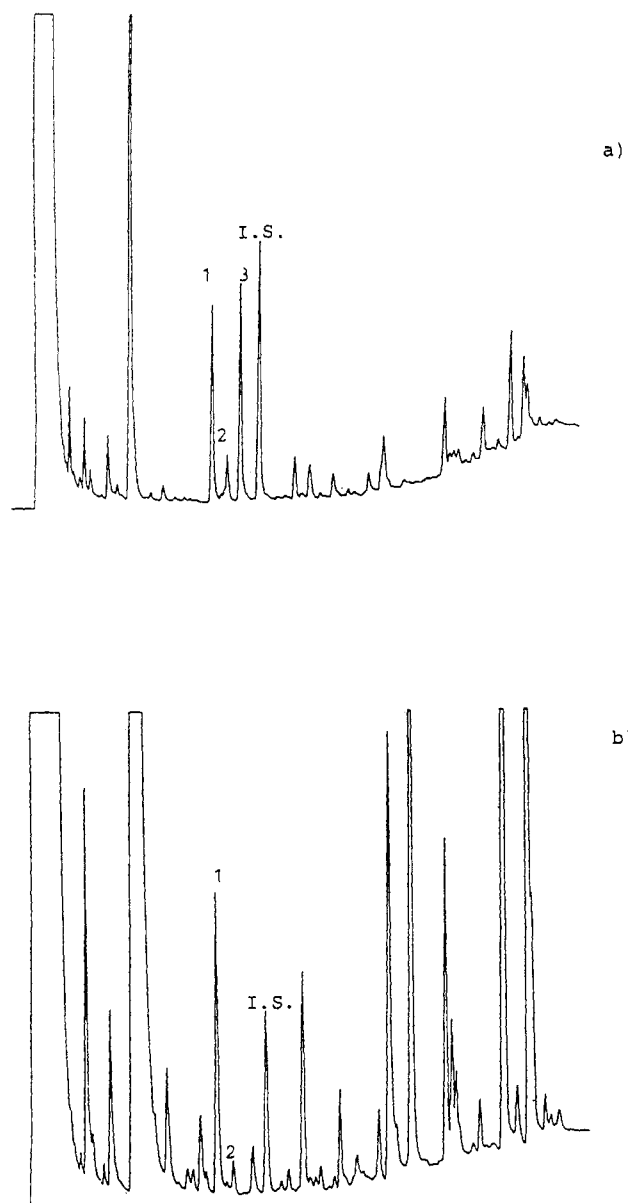


Figure 2. Chromatogram obtained from the Freon extracts of the initial must (a) and the final wine (b). Column and chromatographic conditions as stated in Figure 1. Peak identification: 1, 1-hexanol; 2, (*Z*)-3-hexen-1-ol; 3, (*E*)-2-hexen-1-ol; IS, methyl capilate.

12). As far as (*Z*)-3-hexen-1-ol is concerned, the small amount found in the initial must remains nearly stable during the period of time studied.

Data obtained from the series fermented without SO_2 did not reveal any significant difference with regard to data corresponding to the samples fermented in the presence of sulfur dioxide. Consequently, total elimination of this additive, as recommended by modern vinification technology, would not markedly alter the final composition of alcohols and aldehydes of C_6 chain length during the alcoholic fermentation of sterilized musts.

In order to properly evaluate eventual changes in the concentration of certain C_6 compounds during alcoholic fermentation, individual additions to different portions of synthetic medium were considered as mentioned in Materials and Methods.

Table I gives the three different levels of concentrations in which each compound was added. Values found for the solutes detected after having carried out separately the respective fermentations are also included. Overall errors (including errors affecting extraction, concen-

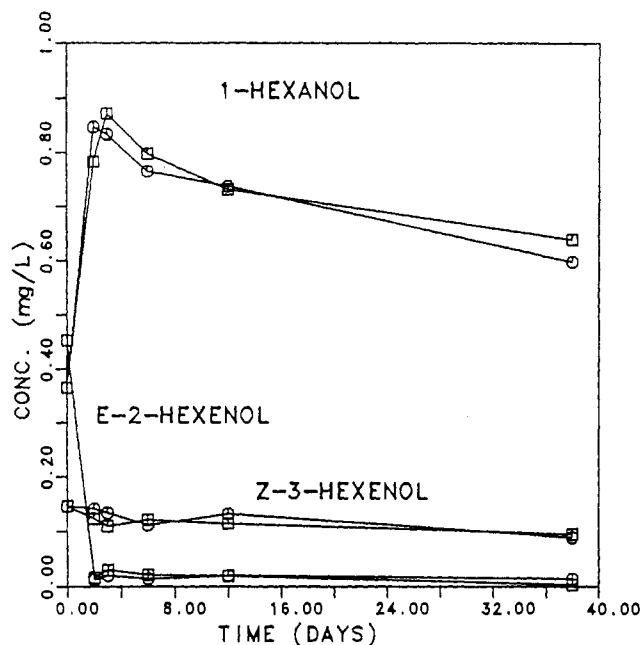


Figure 3. Changes in the composition of 1-hexanol, (*E*)-2-hexen-1-ol, and (*Z*)-3-hexen-1-ol of grape must during alcoholic fermentation by *S. cerevisiae*. Key: O, with SO_2 ; □, without SO_2 .

tration, and chromatographic analysis itself) of quantitative determinations range between 4.7% (for hexyl acetate) and 12.4% (for (*E*)-2-hexen-1-ol), the average overall error being 8.8%. It should be noted that a recovery average (calculated from recoveries corresponding to the three different additions above mentioned) of 1-hexanol close to 78% is obtained in the samples fermented after 1-hexanol was added. Moreover, no significant amounts of aldehydes or alcohols of six carbons are further detected. Analysis of those samples obtained after having added (*Z*)-3-hexen-1-ol or (*E*)-3-hexen-1-ol to each aliquot demonstrated that these alcohols do not exhibit appreciable changes during alcoholic fermentation, and consequently about 90% of each compound is finally recovered. Nevertheless, it is evident that 1-hexanol originating from (*E*)-2-hexen-1-ol and (*Z*)-2-hexen-1-ol is present in the medium. The average recoveries of 1-hexanol are about 49% and 24%, respectively (Table I), and the initial concentration of (*Z*)-2-hexen-1-ol is nearly halved during the fermentation whereas no significant amounts of (*E*)-2-hexen-1-ol were detected in the sample once the fermentation was complete.

From Table I it is also evident that about 24% of hexanal and 34% of (*E*)-2-hexenal are reduced to 1-hexanol while these six-carbon aldehydes do not remain appreciably in the fermented sample.

For comparison, Table I also includes data resulting from the analysis of a control synthetic medium. As can be seen, only small quantities of caproic acid and ethyl caproate were detected. It should be emphasized that data obtained for the last mentioned compounds in samples fermented with the addition of 1-hexanol, (*E*)-2-hexen-1-ol, (*E*)-2-hexenal, or hexanal suggest the use of these compounds for the yeast metabolism.

CONCLUSIONS

The conclusions of this work can be summarized as follows:

The presence of 1-hexanol in wines arises from the 1-hexanol present in the must as well as from reduction of hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*Z*)-2-hexen-1-ol.

The evident attenuation of the leafy grassy odor observed during alcoholic fermentation seems to be due to the corresponding increase in concentration of 1-hexanol since its contribution to the mentioned odor is clearly lower than this one resulting from the unsaturated C₆ alcohols and aldehydes.

(Z)-3-Hexen-1-ol and (E)-3-hexen-1-ol are not metabolized by *S. cerevisiae*, and their concentrations are nearly stable during alcoholic fermentation. Consequently, data found for these compounds in the final wine could be helpful for characterizing the corresponding grape variety and for studying the technological treatment applied to the initial must.

Apart from the well-known action of the oxidoreductase present in grapes, in the reduction of unsaturated aldehydes and alcohols, certain enzymes of *S. cerevisiae* must produce a similar effect during the fermentation.

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Registry No. 1-Hexanol, 111-27-3; (Z)-3-hexen-1-ol, 928-96-1; (E)-2-hexen-1-ol, 928-95-0; (E)-3-hexen-1-ol, 928-97-2; (Z)-2-hexen-1-ol, 928-94-9; hexanal, 66-25-1; (E)-2-hexenal, 6728-26-3.