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Effects of the presence of skins during alcoholic fermentation on the composition of wine volatiles

by

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Der Einfluß des Beerenhautkontaktes während der alkoholischen Gärung auf die Zusammensetzung der Weinaromakomponenten

Z u s a m m e n f a s s u n g : Die Zusammensetzung der flüchtigen und nichtflüchtigen Inhaltsstoffe von Weinen, die aus einem Most der Rebsorte Cencibel unter vier verschiedenen Gärungsbedingungen gewonnen wurden, wird beschrieben. Die Vergärung durch *Saccharomyces cerevisiae* erfolgte mit oder ohne Beerenhäute und mit oder ohne SO$_2$. Die mittels konventioneller Analysenmethoden und auf gaschromatographischem Wege gewonnenen Daten wurden der Faktoren- und Clusteranalyse unterzogen. Bei einigen Komponenten traten signifikante Konzentrationsunterschiede in Abhängigkeit von den Gärungsbedingungen auf.

K e y w o r d s : berry, skin, sulphur, fermentation, wine, flavour, constituent, alcohol, acid, ester, aldehyde, analysis, statistics.

Introduction

In the fermentation of red grape varieties used for red and rosé wine production, must is held in contact with the skins for different periods of time to extract phenolic components for colour. On the other hand, must of white varieties can be eventually maintained in contact with the skins for extracting some compounds, mainly terpenes. In this respect, Cordonnier and Bayonove (1981) and Versini et al. (1981) previously reported the occurrence of high amounts of terpenes in the skin, mainly geraniol, nerol and linalool. Gunata et al. (1985) found that the final concentration of terpenes in wines depended on the skin contact time.

Arnold and Noble (1979) found a significant increase of the aroma of Chardonnay wine following skin contact without remarkable differences in bitterness or astringency. Schmidt and Noble (1983) investigated the differences in white wine flavour produced by varying the skin contact. Lamikanra and Garlick (1987) studied the contribution of grape skin to the composition and quality of Muscadine wines including the variation of the total and the volatile acidity, total phenols and volatile ester concentrations as well as wine colour. According to several authors (Bertrand 1983; Rizzon 1985; Loyola 1988), a decrease in the concentration of ethyl esters was observed, when fermentation of the must was carried out in the presence of grape skins and seeds.

Ramey et al. (1986) studied the effect of temperature during skin contact on Chardonnay must and wine compositions. They found that six carbon compounds related to leafy or herbaceous aroma, namely cis-3-hexen-1-ol, trans-2-hexen-1-ol and 1-hexanol, were developed during skin contact particularly at low temperatures. Benzyl alcohol and 2-phenyl ethanol reached their maximum concentrations when the skin contact was maintained for 30 h. Also, a significant positive correlation between skin contact

time and total phenols in must and the resulting wine, was shown. LOYOLA (1988) detected an increase in the levels of methanol, α-terpineol, nerol and geraniol during the fermentation of Muscat grape must in contact with the skins. He also found significant differences in the contents of some volatile compounds, namely esters and alcohols, as a result of the skin contact time.

Most investigations of skin contact are focused on its effect on the polyphenol composition, while further research about the effect of extended pomace contact time on wine remains still to be done. The aim of this work is to study the effect of skin contact during the fermentation of Cencibel must on composition of the wine volatiles obtained.

Material and methods

Wine samples

Aliquots (300 ml) of a fresh must of cv. Cencibel (La Mancha, Spain) were fermented in 1 l flasks at 22 °C with 2 % (v/v) inocula of 48 h cultures of *Saccharomyces cerevisiae* (var. *ellipsoideus*) provided by the Instituto de Fermentaciones Industriales, CSIC, Spain.

Fermentations were carried out by duplicate in four different series, according to a 2 × 2 factorial design, as follows:

— Series A: Must was fermented without skins and without adding SO₂.
— Series B: Must was fermented without skins but in the presence of SO₂ (200 mg l⁻¹, added as NaHSO₃).
— Series C: Must was fermented with skins (150 g of fresh skins was added prior to fermentation) but in the absence of SO₂.
— Series D: Must was fermented with addition of skins (150 g of fresh skins) and SO₂ (200 mg l⁻¹ of SO₂ added as NaHSO₃).

Analytical procedure

Acetaldehyde, methyl acetate, ethyl acetate, methanol, 2-methyl-1-propanol, 1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol were analyzed by direct injection of 2 µl of wine. A 5 m × 0.85 mm i.d. micropacked column made in our laboratory from deactivated Pyrex tubing loaded with Carbowax 300 + bis-2-ethyl hexyl sebacate (92:8) on desilanized Volaspher A-2, 120—140 mesh (Merck) (4 % w/w) (REGLERO *et al.* 1986) was used.

A 10 m × 0.85 mm i.d. micropacked column made from deactivated Pyrex glass was used for analyzing the medium volatiles fraction including isoamyl acetate, ethyl caproate, hexyl lactate, hexyl acetate, ethyl lactate, 1-hexanol, cis-3-hexen-1-ol, ethyl 3-hydroxybutyrate, 3-ethoxypropan-1-ol, γ-butyrolactone, isovaleric acid, butyric acid, isobutyric acid, ethyl caprylate, linalool, α-terpineol, diethyl succinate, isoamyl lactate, benzyl alcohol and 2-phenyl ethanol. Desilanized Volaspher A-2 (120—140 mesh) was used as solid support with 4 % (w/w) of Igepal CO 880 and Carbowax 20 M (80:20 w/w).

Prior to the chromatographic analysis of the medium volatiles fraction, Freon-11 extracts were prepared according to RAPP *et al.* (1976). A 250 ml sample was continuously extracted for 24 h with 150 ml of freshly bidistilled Freon-11. A 7 µl volume of methyl caprylate (2 % v/v in ethanol) was added as internal standard. A 60 ml sample of the extract was subsequently concentrated at 32 °C to 0.3 ml under a 30 cm Vigreux column. Finally, an aliquot (5 µl) was injected into the GC.

Fatty acids (C₆-C₁₆), ethyl myristate, ethyl palmitate, ethyl stearate and 2-phenyl ethyl acetate, were analyzed by using a 10 m × 0.6 mm i.d. micropacked column loaded

with FFAP on desilanized Volaspher A-2 (120—140 mesh) (3.5 % w/w) (HERRAIZ *et al.* 1988).

A minimum of two injections was carried out in GC analysis per fermentation treatment.

Glycerol, L-lactic acid and L-malic acid were analyzed according to the enzymatic methods of BOEHRINGER (1975). Must and wine total phenols were determined colorimetrically by the Folin-Ciocalteu analysis. The colour intensity was measured as the sum of the absorbancies at 420 and 520 nm. Tartaric acid was quantified spectrophotometrically (REBELEIN 1974). The alcohol degree was measured by distillation and subsequent oxidation with dichromate. The total SO_2 was measured by treatment with NaOH and titration with J_2, and the free SO_2 was measured by titration with J_2.

Statistical methods

The composition of samples was assumed to have two sources of variation, namely the SO_2 (with two levels; presence or absence) and the skins (with two levels: presence or absence). Consequently, data were fitted to a linear fixed-effect model

$$y_{ijk} = \mu + \alpha_i + \beta_j + \delta_{ij} + e_{ijk}$$

where y_{ijk} was the kth observation within the set from level i of the first source of variation (SO_2 factor) and level j of the second source of variation (skins factor); μ was the overall mean, α_i the effect due to the level i of SO_2 factor; β_j the effect due to the level j of skins factors; δ_{ij} the interaction of i, j levels of the ijth set and e_{ijk} the random deviation from the mean of the ijth set. The three statistical hypotheses assayed, in terms of the model, were:

H_1: $\alpha_1 = \alpha_2 = 0$, or, there is no difference between the means of the two levels of SO_2 factor.

H_2: $\beta_1 = \beta_2 = 0$, or, there is no difference between the means of the two levels of skins factor.

H_3: $\delta_{ij} = 0$, i,j $= 1,2$, or, the effects due to SO_2 and skins factors are additive.

Factor analysis (principal component method, from correlation matrix) and cluster analysis (Ward's method, from standardized data) were applied to wine samples.

The BMDP (DIXON 1983) package was used for analysis of variance (BMDP2V program) and for factor analysis (BMDP4M program). Cluster analysis was carried out by using the Clustan program (WISHART 1978). All these programs were run in a CDC CYBER 180/855 computer.

Results and discussion

Table 1 shows the average values obtained from the conventional analysis of the studied samples. The results of the three hypotheses assayed are included. The homogeneity of variances in the four groups was previously tested by Cochran's test.

As was to be expected, the addition of skins produces a remarkable increase of the phenolic compounds and the colour. As can be seen, a slight increase in the contents of the two mentioned parameters is also observed in samples fermented with skins and SO_2 compared with samples fermented without addition of SO_2 but in the presence of skins. As far as glycerol and tartaric acid are concerned, the highest levels are detected in wines obtained from musts which were fermented with skin contact. After fermentation with SO_2 and skin contact, a significant decrease in the total SO_2 content is observed.

Table 1

Mean values (n = 2) and standard error of the means of the conventional parameters in the diffe-
rent groups examined and results of the hypotheses tested

Mittelwerte aus Doppelbestimmungen mit Standardabweichungen der konventionellen Weinpara-
meter in den verschiedenen Versuchsgruppen sowie Gültigkeit der geprüften Hypothesen

Components (mg/l)	Without skins		With skins		Hypotheses tested		
	Without SO$_2$ (A)	With SO$_2$ (B)	Without SO$_2$ (C)	With SO$_2$ (D)	H$_1$	H$_2$	H$_3$
Polyphenols (mg gallic acid/l)	610. ± 24	700. ± 18	2 970. ±75	3 330. ±147		*	
Glycerol	3 730. ± 10	3 620. ± 14	4 930. ±50	4 640. ±100		*	
Total SO$_2$	22.5± 3.1	121.6± 6.4	44.8± 6.4	67.2± 16	*		*
Free SO$_2$	12.8± .00	28.8± 3.2	19.3± .05	22.5± 3.1	*		*
Tartaric acid	470. ±100	410. ± 20	1 640. ±10	2 010. ±300		*	
Lactic acid	40. ± 10	30. ± 10	40. ±10	75. ± 5			
Malic acid	875. ± 35	915. ±250	900. ±95	1 185. ± 5			
Ethanol (% v/v)	16.9± .05	16.2± .30	16.7± .15	16.0± .60			
Colour	3.3± .30	2.5± .05	17.7± .85	19.7± 2.6		*	
pH	4.4± .00	4.3± .00	4.4± .05	4.4± .00			

* Hypothesis rejected (P < 0.05).
H$_1$: $\alpha_1 = \alpha_2 = 0$, or, there are no differences between the means of the two levels of SO$_2$ factor.
H$_2$: $\beta_1 = \beta_2 = 0$, or, there are no differences between the means of the two levels of skins factor.
H$_3$: $\delta_{ij} = 0$, i,j = 1, 2, or, the effects due to SO$_2$ and skins factors are additive.

Table 2 gives the average values obtained by gas chromatography for the different
treatments, together with the results of the three hypotheses tested. The main effects
of the skin contact are increases of methanol, 1-propanol, 2-methyl-1-butanol,
3-methyl-1-butanol, 3-ethoxypropan-1-ol, benzyl alcohol and 1-hexanol, and decreases
of capric acid, caprylic acid, ethyl caprylate and ethyl palmitate. As far as the addition
of sulphur dioxide is concerned, appreciable increases of caproic acid, caprylic acid and
ethyl myristate are observed. In other cases, also significant differences between the
data are observed as a result of the interaction between factors.

Since the concentration of 1-hexanol increases when fermentation is carried out in
the presence of skins, levels of this alcohol in red wines must be higher than in white
wines, as has also been reported by MARTIN-ALVAREZ et al. (1987). The occurrence of
1-hexanol and aldehydes of C$_6$ chain length in wines are considered to be produced
from lipoxygenase and alcohol dehydrogenase catalyzed oxidation of linoleic and lino-
lenic acids; this is known to be a major pathway contributing to the overall wine fla-
vour (DRAWERT 1974). According to other authors, lipoxygenase is mainly located in the
skins, which have also appreciable amounts of insaturated fatty acids (MONTEDORO and
BERTUCCIOLI 1982).

Levels found for benzyl alcohol in the four fermentation series studied seem to
suggest the presence of this alcohol in the skin itself or its generation from some com-
pounds occurring in the skins. On the other hand, the variation of the nitrogen com-
position of the medium resulting from skin contact may result in the increase of the

Table 2

Mean values (n = 2) and standard error of the means of the wine components in the different groups examined and results of the hypotheses tested

Mittelwerte aus Doppelbestimmungen mit Standardabweichungen der Weinkomponenten in den verschiedenen Versuchsgruppen sowie Gültigkeit der geprüften Hypothesen

Components (mg/l)	Without skins		With skins		Hypotheses tested		
	Without SO_2 (A)	With SO_2 (B)	Without SO_2 (C)	With SO_2 (D)	H_1	H_2	H_3
a) Alcohols							
Methanol	37.0 ± 8.4	33.2 ± 1.8	258.8 ± 6.9	249.1 ± .65		*	
1-Propanol	38.6 ± .10	34.8 ± 3.4	61.8 ± .45	56.5 ± .70		*	
1-Hexanol	1.3 ± .05	1.0 ± .15	1.8 ± .05	2.0 ± .00		*	
α-Terpineol	.3 ± .10	.1 ± .00	.1 ± .00	.1 ± .00			
2-Methyl-1-butanol	45.8 ± .25	39.3 ± 1.6	49.5 ± 1.4	50.4 ± .40		*	*
3-Methyl-1-butanol	263.6 ± 1.0	226.4 ± 14.	300.7 ± .30	284.0 ± 2.6	*	*	
Benzyl alcohol	.1 ± .00	tr	.3 ± .00	.4 ± .05		*	*
3-Ethoxypropan-1-ol	.4 ± .05	.3 ± .05	.6 ± .15	1.5 ± .15	*	*	*
2-Phenyl ethanol	63.7 ± 1.5	55.7 ± .45	56.5 ± .15	58.8 ± 1.4			*
cis-3-Hexen-1-ol	tr	tr	tr	tr			
Linalool	tr	tr	tr	tr			
Isobutanol	42.0 ± .85	41.0 ± 2.8	44.0 ± .60	40.3 ± .25			
b) Acids							
Butyric acid	.9 ± .05	.6 ± .00	1.0 ± .10	1.9 ± .00	*	*	*
Lauric acid	1.5 ± .10	.5 ± .00	.5 ± .00	.6 ± .05	*	*	*
Palmitoleic acid	.3 ± .10	.4 ± .00	.3 ± .05	.9 ± .05	*	*	*
Isobutyric acid	2.5 ± .25	1.5 ± .00	1.5 ± .25	3.8 ± .25	*	*	*
Caprylic acid	1.7 ± .00	2.5 ± .25	1.3 ± .10	2.0 ± .15	*	*	
Caproic acid	1.4 ± .00	1.8 ± .15	1.5 ± .05	2.0 ± .05	*		
Capric acid	.6 ± .05	.7 ± .10	.4 ± .05	.5 ± .10		*	
Isovaleric acid	2.8 ± .10	1.9 ± .15	1.8 ± .25	2.7 ± .05			*
Palmitic acid	.4 ± .05	.3 ± .05	.3 ± .05	.3 ± .10			
c) Esters							
Ethyl lactate	.5 ± .05	.4 ± .05	.8 ± .10	1.2 ± .05		*	*
Ethyl stearate	.2 ± .00	tr	tr	tr			
Isoamyl acetate	.3 ± .00	1.2 ± .20	.1 ± .00	.1 ± .00	No hom. var.		
Ethyl 3-hydroxy-butyrate	.9 ± .10	.3 ± .00	.7 ± .10	1.4 ± .05		*	*
γ-Butyrolactone	43.3 ± 5.5	7.4 ± .85	16.9 ± 1.6	24.6 ± 4.8	*		*
Ethyl caprylate	1.6 ± .25	1.8 ± .15	.9 ± .00	.9 ± .05		*	
Diethyl succinate	1.0 ± .15	.5 ± .00	.9 ± .05	.9 ± .05			*
Hexyl acetate	tr	tr	tr	tr			
Ethyl myristate	.1 ± .00	.3 ± .05	tr	.3 ± .15			
Ethyl palmitate	.1 ± .00	.1 ± .00	tr	tr			
Ethyl acetate	78.3 ± .20	85.8 ± 3.9	93.4 ± 6.4	81.3 ± 1.1			
Isoamyl lactate	.1 ± .00	.1 ± .00	.1 ± .00	.2 ± .05			
Ethyl caproate	.7 ± .10	.8 ± .10	.6 ± .05	.6 ± .00			
Methyl acetate	7.3 ± 1.6	7.8 ± 1.8	4.4 ± 1.7	4.8 ± .10			
2-Phenyl ethyl acetate	.1 ± .00	.1 ± .00	.1 ± .00	.2 ± .05			
d) Aldehydes							
Acetaldehyde	18.4 ± .65	145.7 ± 13.	7.8 ± .55	18.7 ± .10	No hom. var.		

* Hypothesis rejected (P < 0.05), tr = traces (< 0.1 mg/l), H_1, H_2 and H_3 as in Table 1, No hom. var. = No homogeneity variances.

higher alcohol levels which is shown in Table 2. Also different contents in fatty acids and esters are due to certain changes in the conditions of fermentation, namely to the presence of skins in the medium. It is interesting to point out that a significant decrease in the content of acetaldehyde and isoamyl acetate is followed when the fermentation is carried out in the presence of SO_2 and skins compared with the fermentation accomplished in the presence of SO_2 and the absence of skins.

Table 3 shows the results of factor analysis (principal component method) applied to the samples analysed ; the corresponding tridimensional representation is given in Fig. 1. As can be seen, the three first factors explain 82.7 % of the total variance. The first factor is related to the skin contact time and the second factor allows the differentiation of wines included in series A and B, while series C and D can be distinguished from the third principal component.

Table 3

Results of factor analysis applied to the components of wines listed in Table 2

Ergebnisse der Faktorenanalyse der in Tabelle 2 aufgelisteten Weinkomponenten

Factor	Variance explained (%)	Cumulative proportion (%)	Variables more correlated	Loading
1	35.16	35.16	Caproic acid	.947
			Ethyl caprylate	.943
			Ethyl caproate	.940
			1-Propanol	−.879
			Methanol	−.875
			3-Methyl-1-butanol	−.866
			1-Hexanol	−.834
			Caprylic acid	.832
2	25.60	60.76	γ-Butyrolactone	.965
			Lauric acid	.928
			2-Phenyl ethanol	.907
			Ethyl stearate	.902
			α-Terpineol	.853
			Isovaleric acid	.841
3	21.95	82.71	Palmitoleic acid	.966
			Ethyl myristate	.865
			3-Ethoxypropan-1-ol	.854
			Butyric acid	.852

The results of the application of cluster analysis to the wines, using the 37 variables already mentioned in Table 2, are shown in Fig. 2 a. The Euclidean distance was taken as a measure of the proximity between two samples. It is clear that the main variation between groups is due to the presence or the absence of skins during the fermentation, but different groups are also obtained depending upon whether SO_2 is added or not. However, the presence of skins reduces those differences resulting from the use of SO_2. In this respect, we previously reported that the presence of SO_2 gives rise to wines of different composition since its addition produces a selection of the microorganisms which are present in the medium (HERRAIZ et al. 1989).

Dendrograms obtained from cluster analysis of wines, using as variables alcohols, esters and acids separately, are shown in Fig. 2 b through d. The different groups obtained for each set of variables show the most remarkable changes occurring in some compounds depending upon the conditions under which fermentation was carried out. Levels of alcohols change mainly with the presence or the absence of skins, while the addition of SO₂ affects the concentration of esters in wines. The presence of SO₂ and skins can modify the content of acids in wines.
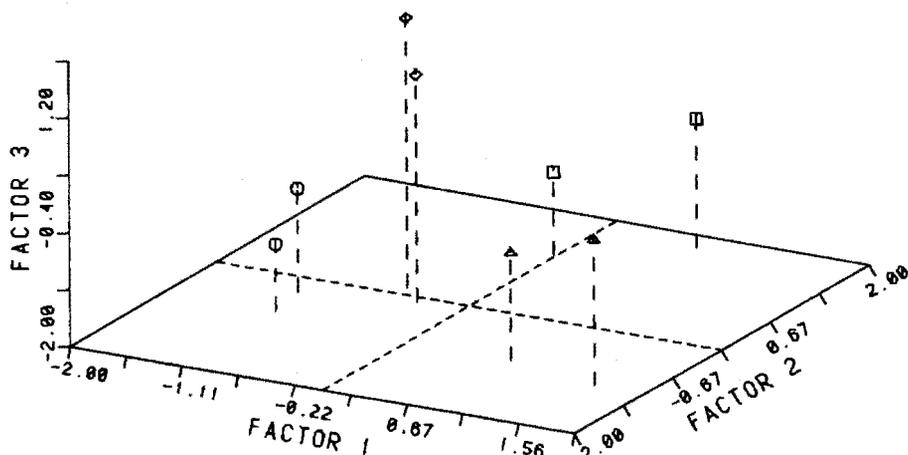
Fig. 1: Tridimensional presentation of the wines resulting from the application of principal component analysis (for further details see text). □ = Without SO₂ and without skins; ○ = without SO₂ and with skins; △ = with SO₂ and without skins; ◇ = with SO₂ and with skins.

Dreidimensionale Darstellung der Weine aufgrund der Hauptkomponentenanalyse. □ = Ohne SO₂, ohne Beerenhäute; ○ = ohne SO₂, mit Beerenhäuten; △ = mit SO₂, ohne Beerenhäute; ◇ = mit SO₂, mit Beerenhäuten.

From our results it seems to be clear that the fermentation in the presence of skins produces an increase in the level of alcohols and a decrease of several ethyl esters and medium chain length fatty acids, which is generally considered as undesirable for the overall wine flavour. If the fermentation is carried out with SO₂ added, an increase in the contents of medium chain length fatty acids is also produced and consequently an inhibition of the alcoholic fermentation can be followed (GENEIX et al. 1983). On the other hand, the presence of skins and SO₂ during winemaking gives rise to a significant increase of several short chain length fatty acids which eventually can diminish the overall wine quality.

It should be noticed that in the media fermented without skins and without SO₂, a compensatory balance of alcohols, ethyl esters and fatty acids is achieved. Therefore, the adequate control of red wine quality seems to demand a careful evaluation of the vinification practices applied for red grape varieties.

Conclusions

An increase in the concentration of some alcohols and a decrease in several ethyl esters and medium chain length fatty acids are reported as the most significant changes occurring in the volatile composition of wines obtained from cv. Cencibel, provided that the fermentation is carried out in the presence of skins.

Summary

The composition of volatile and non-volatile constituents of wines resulting from the fermentation of a must of cv. Cencibel with *Saccharomyces cerevisiae* under differ-
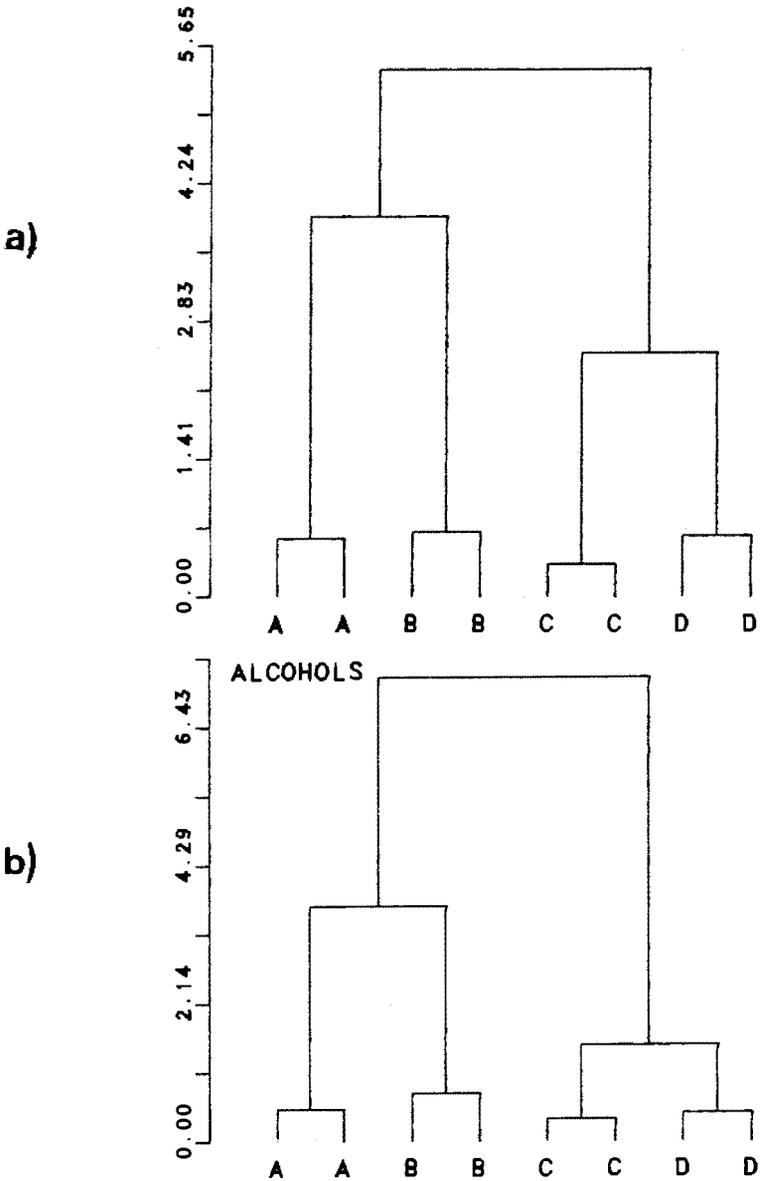

Fig. 2: Dendrograms obtained for the wines using the 37 variables listed in Table 2 (a) as well as alcohols (b), acids (c) and esters (d) separately (for further details see text).

ent conditions is reported. Four different series of fermentations, with or without skins and in the presence or absence of sulphur dioxide, were carried out. Factor and cluster analyses were applied to data obtained by conventional methods of analysis and gas chromatography. There were significant differences in some compounds depending upon the conditions of fermentation.
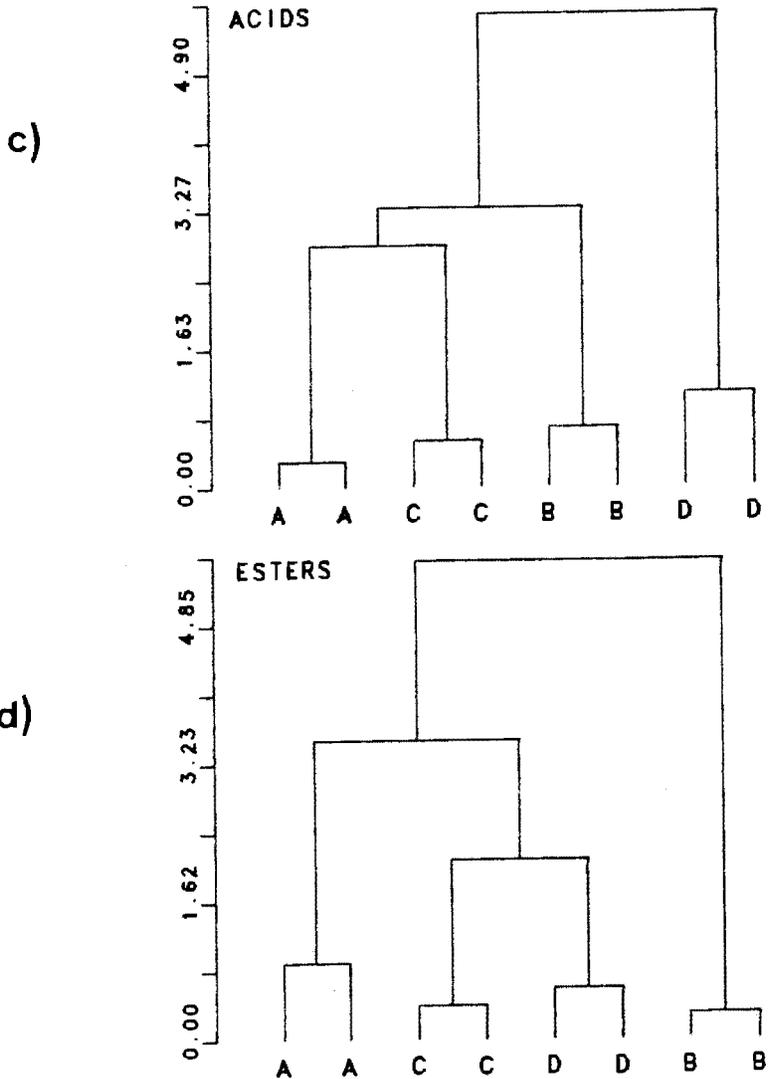

Abb. 2: Dendrogramme der Weine bei Verwendung aller 37 Komponenten der Tabelle 2 (a) sowie einzelner Verbindungsgruppen: Alkohole (b), Säuren (c), Ester (d).

Acknowledgements

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