Food Chemistry **31** (1989) 177–187

The Influence of Yeasts on Certain Non-volatile Components of Wine

J. Barcenilla, I. Estrella, C. Gómez-Cordovés, T. Hernández & L. Hernández

Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva, 3, 28006 Madrid, Spain

(Received 16 December 1987; revised version received and accepted 11 April 1988)

ABSTRACT

Industrial grape must from white Airén grapes was fermented using three yeast species, one for each of the three traditional stages of fermentation. In this work pure and mixed cultures were used. TLC, HPLC and GLC were used to analyse the flavonoid and non-flavonoid phenol, polyalcohol and residual sugar compositions.

Differences in concentrations of phenolic compounds, polyalcohols and sugars, were found to vary depending on the yeast species, in pure or mixed fermentations.

INTRODUCTION

Microbial activity on polyphenolic compounds has been studied by different authors during the past twenty years, but only recently has the activity of yeasts on non-flavonoid compounds been described (Feuillat *et al.*, 1981; Gómez-Cordovés & Khayyat, 1981; Ough & Winger, 1982; Estrella *et al.*, 1983; Kontek & Kontek, 1984). The present study examines specific aspects of such activity and its possible influence on the final properties of wine. Industrial grape must prepared from Airén grapes was fermented in the laboratory and the compounds directly involved with wine flavour, such as flavonoid and non-flavonoid phenols, polyalcohols and sugars, were analysed. The presence of certain phenols which may be responsible for

Food Chemistry 0308-8146/89/$03·50

certain unwanted flavour defects like bitterness (Salagoïty-Auguste & Bertrand, 1984), was also determined.

Analyses were carried out by employing a variety of chromatographic techniques, i.e. thin-layer chromatography (TLC), higher performance liquid chromatography (HPLC) and gas–liquid chromatography (GLC).

MATERIALS AND METHODS

Must-Juice, without skins, was clarified by centrifugation (3000 rpm, 4 min).

Control-Must was fermented using the microorganisms naturally present.

Yeast used was from the Instituto de Fermentaciones Industriales's own stock:

(A) *Kloeckera apiculata*, strain 1059. Yeast present in the early stages of fermentation of all Spanish grape musts; in hot regions its sporal form is used.

(B) *Torulaspora rosei*, strain 789, currently known as *Torulaspora delbrueckii*, is a yeast present during the second stage, and is considered to yield a pure fermentation.

(C) *Saccharomyces cerevisiae* var. *ellipsoideus* strain 87, now regarded as the equivalent of *Saccharomyces cerevisiae* according to the latest taxonomic listing by Kreger-van Rij (1984), is a yeast that is always present in the third and final stages of fermentation in all grape musts from all Spanish wine-producing regions studied.

Sampling:

(I) First sample collected during bubbling fermentation (after 6 days)

(II) Second sample collected at the end of fermentation (once weight remained constant (after 20 days)).

Chromatography

Commercially available standards were from Sigma, Fluka, Aldrich and Applied Science. Samples were prepared from 100 ml of wine as per the method of Diez & Gómez-Cordovés (1977).

TLC: Bidimensional Chromatography was in cellulose MN-300 and like solvents: 1st formic acid/water (2:98) and 2nd isopropanol/ammonium hydroxide/water (8:1:1) (Diez & Gómez-Cordovés, 1980).

Visualisers: Observation carried out under UV light at 254 and 360 nm; spraying by basic lead acetate; diazotised *p*-nitroaniline; 0·4% catechin in acetone/sulphuric acid 25% (1:1); 1% *p*-vanillin in hydrochloric acid 70%. The action of the last three reagents was effected at 40°C.

HPLC: Flavonoid phenols were done according to the method of Alonso *et al.* (1986) modified by using a stainless steel Novapak C_{18} column (150 × 3·9 mm), 5 μ. The solvent was methanol/acetic acid/water (37·5:5:67·5); solvent flow rate, 0·7 ml/min. The detector wavelength was 365 nm.

Non-flavonoid phenols were done according to the method of Hernández & Dorronsoro (1984), modified by using a stainless steel Ultrasphere-ODS column (250 × 3·9 mm) 5 μ. Solvent A was: water/acetic acid (98:2); solvent B was: water/methanol/acetic acid (68:30:12).

The elution gradient was:

t (min)	*Flow (ml/min)*	*% A*	*% B*
0	1·0	100	0
3	1·0	100	0
5	1·0	50	50
10	1·1	40	60
20	1·2	30	70
22	1·2	25	75
25	1·2	20	80
30	1·2	15	85
50	1·2	15	85

Curve profile 5. Detection 280 and 340 nm.

GLC: Polyalcohols and sugars were done according to the methods of Santa-Maria & Olano (1985) and Hernández & Gómez-Cordovés (1986). Capillary column 25 × 0·2 mm inside diameter with OV 101 as the stationary phase. The oven temperature was 200 °C for 9·5 min which was raised to 250 °C at rate of 30 °C a minute.

PROCEDURES

The bottled must (500 ml per bottle) was then inoculated with 20 ml of yeast starter culture prepared from three yeasts. The starter culture used for seeding had been incubated at 20 °C, and the yeasts were in the logarithmic growth phase.

The three yeasts were selected for use in the experiment because each represented a precise stage in the spontaneous fermentation of grape musts. Fermentation has traditionally been divided into three stages, an initial stage in which fermentation is not yet perceptible, a second stage in which fermentation is active and bubbling and a final stage in which fermentation slows and comes to an end.

TABLE 1
Compounds in Must

Fructose	44·12 g/litre
Glucose	42·97 g/litre
Erythritol	0·30 mg/litre
Arabitol	—
Mannitol	—
Sorbitol	0·11 mg/litre
Inositol	0·57 mg/litre
Caffeic acid	0·49 mg/100 ml
Cl_s Ferulic acid	0·17 mg/100 ml
Trans Ferulic acid	0·14 mg/100 ml
p-Coumaric acid	0·48 mg/100 ml
p-Hydoxybenzaldehyde	—
Tyrosol	—
Tryptophol	—
Myricetin	47·1 μg/100 ml
Quercetin	41·9 μg/100 ml
Kaempherol	0·8 μg/100 ml
Isorhamnetin	—

The musts (see Table 1) were inoculated as follows: pure fermentation in three of the bottles A, B and C; mixed fermentation in another three bottles AB, AC and BC and one bottle containing all three species ABC. Fermentation using the microorganisms naturally present in the must was also carried out as a control in one bottle.

After inoculation, the bottles were sealed with Müller valves and weighed, after which they were incubated at 20°C. Fermentation was followed daily by recording the weight loss undergone by the bottles.

RESULTS AND DISCUSSION

Fermentation

The bottles inoculated with *K. apiculata* had the lowest weight loss. The bottles seeded with *S. ellipsoideus* and the combination of all three yeasts had weight losses similar to that of the control bottle.

Non-flavonoid phenolic compounds

The compounds identified appear on the chromatographic plate shown in Fig. 1 and in the chromatograms presented in Fig. 2. Compounds that were

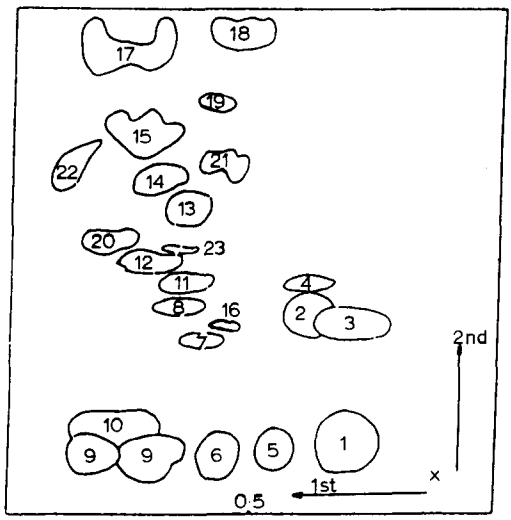

Fig. 1. Solvent: 1st: Formic acid/water (2:98), 2nd: Isopropanol/ammonium hydroxide/water (8:1:1). 1. Caffeic acid. 2. Esculetin. 3. *trans*-Ferulic acid. 4. *trans-p*-Coumaric acid. 5. Gallic acid. 6. Protocatechuic acid. 7. Vanillic acid. 8. *p*-Hydroxybenzoic acid. 9. Chlorogenic acid. 10. Cinnamic acid; tartaric acid esters. 11. *cis* Ferulic acid. 12. *cis p*-Coumaric acid. 13. Syringaldehyde. 14. *p*-Vanillin. 15. *p*-Hydroxybenzaldehyde. 16. α-Resorcylic acid. 17. Tyrosol. 18. Tryptophol. 19. Salicylic acid. 20. *p*-Hydroxyphenylacetic acid. 21. Gentisic acid. 22. *p*-Hydroxyphenylpropionic acid. 23. *m*-Hydroxybenzoic acid.

quantified have been numbered in the figures. Quantification was carried out by comparison with standard solutions of known concentrations chromatographed under the same conditions. The results appear in Table 2 and in Figs 3 and 4 and are expressed in mg/100 ml of wine.

Concentrations of tyrosol (Ty) and tryptophol (Tr), (Fig. 3), were higher with sample II. Production of both alcohols by the B yeast was apparently

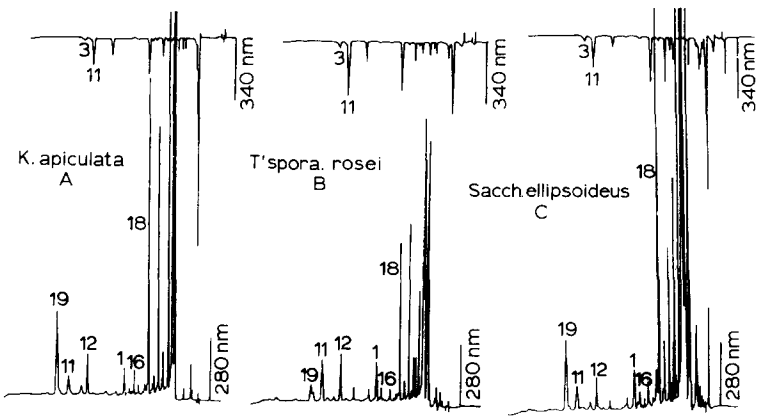

Fig. 2. HPLC chromatograms obtained from pure fermentation.

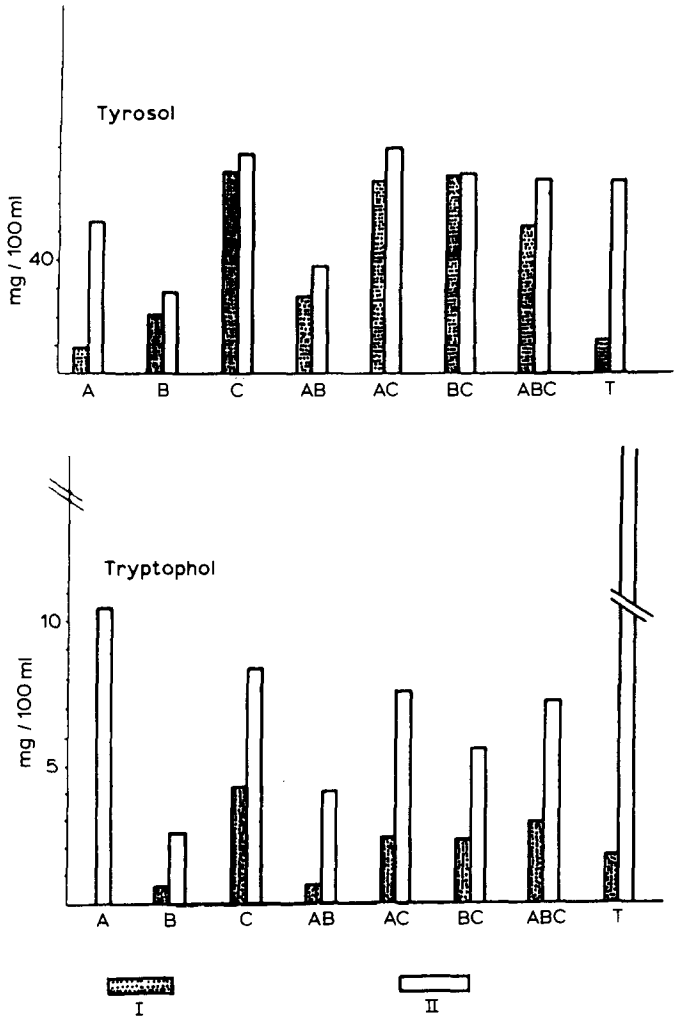

Fig. 3. Histogram of tyrosol and tryptophol.

lower, and this yeast would also seem to inhibit production by the other yeast. Tr production was greater with A yeast, whereas Ty production seemed to take place chiefly while the C yeast was active.

The trend for *p*-hydroxybenzaldehyde (pOHBL) (Fig. 4) was opposite to that of all the other compounds studied, in that its level decreased in sample II. This could be attributable to two separate yet overlapping effects; first, possible oxidation to the corresponding acid and, secondly, direct action by the yeasts, which may have used it as a metabolite in the production of other compounds.

Of the three cinnamic acids, the most abundant was caffeic acid (Caf),

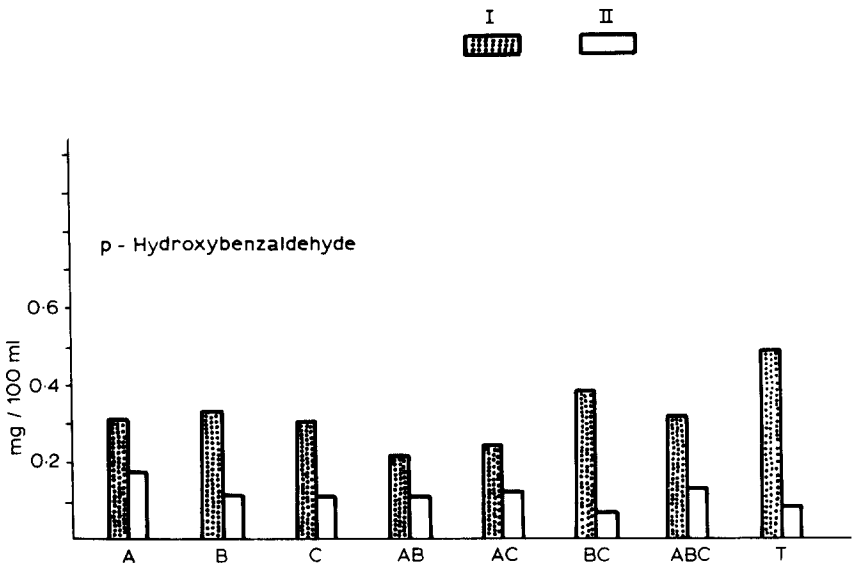

Fig. 4. Histogram of *p*-hydroxybenzaldehyde.

followed by *p*-coumaric acid (Coum), and lastly, ferulic acid *cis* and *trans* (Fer-c, Fer-t). All three (Table 2) increased during fermentation. The amounts of caffeic acid produced by the three yeast species were quite similar. This was not the case for the other two cinnamic acids, which appeared to be produced in greater quantities by B, and this effect was observed in all the samples in which this yeast species was present.

TABLE 2
Phenolic Compounds (in mg/100 ml of Wine)

Compound and sample		*Yeast*							
		A	*B*	*C*	*AB*	*AC*	*BC*	*ABC*	*Control*
Caf	I	0·87	1·25	1·08	1·21	0·91	0·83	1·70	0·49
	II	1·21	1·73	1·77	1·73	1·67	1·25	1·84	1·18
Fer-c	I	0·38	0·81	0·49	0·70	0·26	0·62	0·94	0·13
	II	0·47	1·11	0·60	1·30	0·47	0·62	1·14	0·13
Fer-t	I	0·14	0·32	0·28	0·43	0·07	0·36	0·43	0·07
	II	0·14	0·42	0·25	0·43	0·21	0·32	0·43	0·14
Coum	I	0·61	0·67	0·54	0·82	0·26	0·54	0·74	0·04
	II	1·06	1·36	0·80	1·41	0·60	0·89	1·15	0·26

Caf (Caffeic acid); Fer-c (*cis*-Ferulic acid), Fer-t (*trans*-Ferulic acid); Coum (*p*-Coumaric acid).

TABLE 3
Flavonoid Aglycones (in μg/100 ml of wine)

Compound and sample		*Yeast*							
		A	*B*	*C*	*AB*	*AC*	*BC*	*ABC*	*Control*
Myr	I	31·4	31·4	24·3	63·6	25·0	28·5	18·6	8·5
	II	60·0	52·8	24·3	77·1	48·6	50·0	57·1	0·9
Quer	I	43·5	12·9	50·0	23·4	16·1	9·7	10·5	17·7
	II	30·8	20·9	16·1	23·4	9·7	9·7	11·3	0·7
Kaem	I	7·7	2·3	0·7	4·5	tra	tra	tra	0·8
	II	tra	tra	tra	—	—	tra	tra	—
I-ram	I	—	—	—	—	tra	—	—	—
	II	tra	tra	—	—	—	—	—	—

Myr: Myricetin; Quer: Quercetin; Kaem: Kaempherol; I-ram: Isorhamnetin; Tra: Traces.
—: not detected.

Flavonoid phenolic compounds

The results appear in Table 3, expressed in μg/100 ml of wine.

In sample II, the concentration of myricetin is higher with the A yeast and rises from sample I to sample II. However, quercetin decreased during fermentation and this action is higher by the C yeast.

Kaempherol was almost entirely absent from the final fermented product, though its initial concentration was also low.

Polyalcohols

The results are presented in Table 4.

The largest amounts of erythritol, a C_4 polyalcohol, were produced by the A yeast and the smallest by the C yeast. This confirms the literature report to the effect that it is formed in the initial stage of fermentation by the A yeast. When the actions of A, B and C yeasts overlapped, production of this polyalcohol decreased and the C yeast exhibited the strongest inhibitory effect.

The B yeast produced much higher amounts of arabitol, up to 576 mg/litre, and production was even augmented by overlapping activity by the A yeast in sample II, whereas production by the C yeast was very low, with values as low as 35 and 10 mg/litre. The C yeast had a strong inhibiting effect on the production of this compound.

TABLE 4
Polyalcohols (in mg/litre of wine)

Compound and sample		*Yeast*							
		A	*B*	*C*	*AB*	*AC*	*BC*	*ABC*	*Control*
Er	I	102	35·9	88·3	23·9	123	71·9	67·3	34·7
	II	292	161	120	171	132	131	116	74·1
Ar	I	91·6	229	35·5	208	42·9	116	248	70·5
	II	86·7	577	9·6	769	13·7	151	384	60·2
Man	I	47·8	—	56·4	66·1	39·2	46·4	72·1	5·5
	II	49·7	155	82·5	152	75·5	103	109	38·5
So	I	—	56·3	30·0	61·2	23·4	36·8	55·2	9·0
	II	68·8	277	30·3	241	31·3	79·9	96·1	28·6
In	I	419	433	407	547	482	370	450	355
	II	536	418	441	474	448	400	441	379

Er: Erythritol; Ar: Arabitol; Man: Mannitol; So: Sorbitol; In: Inositol.

The differences in the inositol contents among the yeast species were not large.

Sugars

Analysis of the sugars, glucose and fructose, was performed together with that of polyalcohols, and results appear in Table 5, expressed in g/litre. This Table shows a substantial difference in sugar decrement for both sugar types, between samples I and II, as expected, since the action time of the yeast is longer in sample II. In samples A, B and C, the different decreases in sugars are related to the yeast species because each one has its own fermentation

TABLE 5
Sugars (in g/litre of wine)

Compound and sample		*Yeast*							
		A	*B*	*C*	*AB*	*AC*	*BC*	*ABC*	*Control*
Fructose	I	18·03	20·91	16·16	15·99	19·76	18·99	14·21	21·26
	II	13·31	6·50	0·12	7·75	0·12	0·11	0·18	0·09
Glucose	I	20·94	24·65	2·50	19·25	2·19	3·49	7·90	21·59
	II	18·84	1·53	0·11	2·68	0·08	0·06	0·06	0·06

TABLE 6
Ethanol Contents at the End of Fermentation

Sample	A	4·6
Sample	B	9·8
Sample	C	10·3
Sample	AB	9·5
Sample	AC	10·4
Sample	BC	10·4
Sample	ABC	10·6
Sample control		10·6

power. Yeast C, catalogued in the bibliography as one of the most fermentative species, is the one which produces the highest decrease. Yeast A, active in the first phase of fermentation and with low fermentation power, is the one which consumes the least amount of sugar.

In samples with two or three yeasts, consumption of sugars depends on the most fermentative yeast of those present.

We can also observe, in Table 5, the different selectivity for sugars by each yeast. Ethanol contents after fermentation are listed in Table 6.

ACKNOWLEDGEMENT

The authors wish to acknowledge financial support of the Consejo Superior de Investigaciones Cientificas (CSIC) for this research.

REFERENCES

Alonso, E., Estrella, I. & Revilla, E. (1986). La separación de flavonoles por HPLC y sus aplicaciones en enología. *XXI Reunión Bienal de la R.S. Española de Química*. Santiago de Compostela. España. p. 560.

Diez, C. & Gómez-Cordovés, C. (1977). Aportación al estudio de los vinagres españoles. IV. Sustancias fluorescentes y polifenoles totales. *Rev. Agroquim. Tecnol. Aliment.*, **17**, 353–62.

Diez, C. & Gómez-Cordovés, C. (1980). Compuestos fenólicos de pequeño peso molecular. Su influencia en la calidad de los vinagres. *Rev. Agroquim. Technol. Aliment.*, **20**, 247–56.

Estrella, I., Hernández, T. & Diez, C. (1983). Evoluzzione dei composti fenolici di basso peso moleculare durante la maturazione dei vini di Jerez. *Vignevine* (1–2), 33–8.

Feuillat, M., Radix, A., Dubois, P. & Dekimpew, J. (1981). Elevage du vins de Bourgogne en fûts de chêne. Contribution à l'étude des composés phénoliques et des composés volatils au cours de cet élevage. *Vignes et Vins*, **299**, 5–10.

Gómez-Cordovés, C. & Khayyat, N. (1981). Efecto de las levaduras sobre los aldehidos y acidos fenolicos presentes en mostos de uva españoles. *Anal. Bromatol XXXIII*, (1) 143–8.

Hernández, T. & Dorronsoro, J. L. (1984). Comportement des composés phénoliques mesures à differentes longueurs d'onde dans l'analyse par C.L.H.P. *Bull. Liaison. G. Polyphenols*, **12**, 587–90.

Hernández, L. & Gómez-Cordovés, C. (1986). Determinacín rapida de polialcoholes y azucares en vinos por cromatografia gas-líquido. *La Sem. Vitiv.* (2102), 4673–5.

Kontek, A. & Kontek, A. (1984). Relations between some phenolic compounds and microorganisms playing an important role in enology. *Bull. Liaison G. Polyphenols*, **12**, 100–7.

Kreger-Van Rij, N. J. W. (1984). *The Yeast: A Taxonomic Study*. Elsevier Science Publisher, B. V. Amsterdam.

Ough, C. & Winger, C. (1982). Changes in non-volatile compounds and extracts of wines due to yeast species and fermentation temperature. *S. Afr. J. Enol. Vitic.*, **3**(1), 17–21.

Salagoïty-Auguste, H. & Bertrand, A. (1984). Wine phenolic analysis of low molecular weight components by high performance liquid chromatography. *J. Sci. Food Agric.*, **35**, 1241–7.

Santa-Maria, G. & Olano, A. (1985). Differences in polyols content among fermentations of the same must with several yeasts. *Biotechnology Letters*, **7**(4), 229–34.

Stahl, E. (1969). *Thin-Layer Chromatography*. Springer-Verlag, Berlin.