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Tryptophol Content of Young Wines Made from Tempranillo, Garnacha, Viura and Airén Grapes

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ABSTRACT

The characteristics of the tryptophol present in young wines made in the laboratory from Tempranillo, Garnacha, Airén and Viura grapes were determined by thin-layer chromatography (TLC). The concentration of this substance in the wines, obtained by means of high performance liquid chromatography (HPLC), was found to be statistically significant in both the red and white wines.

INTRODUCTION

The results of applying TLC to Spanish wines from different regions indicated the presence of a series of unidentified substances forming during fermentation. The apparent concentrations varied in the different types of wine and hence could be linked to certain characteristics affecting their organoleptic properties and quality.

One of the substances exhibited chemical and chromatographic behaviour resembling that of alcohols. However, because it was not a substance commonly encountered in the literature, such as vanillol, *p*-hydroxy benzylalcohol, or tyrosol, other possibilities had to be considered.

Tryptophol has been mentioned as an alcohol present in non-vintage wines by Ribéreau-Gayon & Sapis (1965) and Sapis & Ribéreau-Gayon

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(1969*a*, *b*), who noted that the processes leading to its formation had not been clearly ascertained and that its presence in red wines had been corroborated, it being found less frequently in white wines. Nykänen *et al.* (1966) identified it using infra-red and gas chromatographic techniques. Salagoïty-Auguste & Bertrand (1984) quantified the tryptophol content of red wines using HPLC.

MATERIALS AND METHODS

Wines

Twenty-eight samples from wines made in the laboratory to preclude any characteristics that might arise from regional wine-making procedures and to ensure that only a single variety of grape was used in each; eleven of the wines were made from white Airén and Viura grapes and seventeen from red Tempranillo and Garnacha grapes. The musts were obtained by lightly pressing the grapes.

Wine-making procedures

A single strain of yeast, *Saccharomyces ellipsoideus*, was used for all the wines and the temperature in the fermentation chamber was kept at 28°C.

Sample preparation

A hundred millilitres of wine were concentrated to 25 ml *in vacuo* at 35°C. Four extractions of the concentrate were made using 10 ml of ethyl ether each time and, after drying with anhydrous Na_2SO_4 for 1 h, the extract was desiccated *in vacuo* at 35°C. The residue was taken up with 1 ml of 50% methanol–water solution (Díez *et al.*, 1980).

TLC

Plates with MN300 cellulose, microcrystalline cellulose, and Silica gel G.

Solvents and developers

Given in Tables 1 and 2.

TABLE 1
Tryptophol: R_f Values Obtained Using Different Support Materials and Solvents

Solvent	*Support material*	R_f
2% Formic acid	MN300 cellulose	0·59
Isopropanol–ammonium hydroxide–water (8:1:1)	MN300 cellulose	0·90
2% Acetic acid	MN300 cellulose	0·60
20% Potassium chloride	MN300 cellulose	0·41
n-Butanol–acetic acid–water (4:1:5)	MN300 cellulose	0·98
n-Butanol–acetic acid–water (4:1:5)	Silica gel G	0·98
n-Butanol–acetic acid–water (6:1:2)	MN300 cellulose	0·98
Benzene–dioxane–acetic acid (90:25:4)	Silica gel G	0·48

TABLE 2
Tryptophol: Colour Obtained using Different Developers

Developer	*Colour*
360 nm ultra-violet fluorescence	—
254 nm ultra-violet fluorescence	Bright violet, ammonia-vapour enhanced
Basic lead acetate fluorescence	Light at 360 nm, dark at 254 nm
Diazotized *p*-nitroaniline + 15% sodium carbonate	White on yellow background
Vanillin hydrochloric acid	Purple
Catechin	Pink–purple
2,4-dinitrophenylhydrazine	Light brown
Hydrazinium sulphate	Light brown
Diazotized benzidine	Pale yellow
2N sulphuric acid	Slightly pink
Ferric chloride + potassium ferricyanide	Blue
Folin–Ciocalteu reagent	Blue
α–α′-dipyridyl	—
Iodine	—
Ehrlich reagent (*p*-dimethylaminobenzaldehyde)	Purple
Ehrlich reagent (*p*-aminobenzaldehyde)	Brown
Cinnamaldehyde	Yellow–brown

HPLC

Waters Associates liquid chromatograph equipped with an M-U6K injector, two M-6000A pumps, an M-660 gradient programmer and an M-440 detector. An RCM-100 radial compression system with a Radial Pak A reversed phase column and precolumn filled with Bondapak C_{18}/Corasil. The method of Hernández & Sánchez (1980) was employed:

Mobile phase. Solvent A: acetic acid/water (2:98, v/v). Solvent B: acetic acid/methanol/water (2:30:68, v/v/v). Flow rate: 2·5 ml/min.
Gradient: 0·0% B–70% B in 25 min.
Detection at 280 and 340 nm.
Duration of chromatogram: 35 min.
Each sample injected three to five times.

Mass spectrum

Perkin–Elmer RMU-6MG; peaks recorded at 70 eV.

Statistical analysis

By difference of means applying Student's *t*-test and by analysis of variance (AOV).

RESULTS AND DISCUSSION

Since apparently an alcohol was involved, its indole structure was demonstrated from reactions with vanillin, Ehrlich's reagent and cinnamaldehyde, and it was then possible to verify that the substance was indeed tryptophol by comparing results with a commercially available standard of its chromatographic behaviour (Tables 1 and 2) and of its ultraviolet (peak at 282 nm) and mass spectra [161(M^+ 20%), 130(100%), 77(5%), 103(5%)].

Its presence was corroborated in both the red and white wines studied. Quantification was effected using HPLC (Fig. 1). In the conditions employed, at 280 nm the chromatographic peaks for tryptophol and for *cis*-ferulic acid overlapped; the former had no response at 340 nm, while the latter did. Therefore, using patterns for mixtures of varying

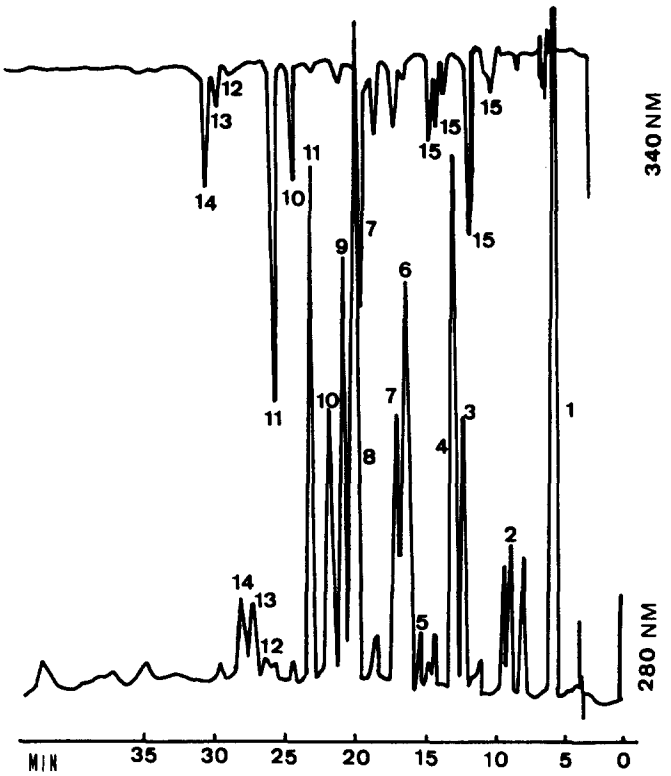

Fig. 1. Garnacha wine. Compounds identified by means of HPLC. 1, Gallic acid. 2, Protocatechuic acid. 3, *p*-Hydroxybenzoic acid. 4, Tyrosol. 5, Catechin. 6, Vanillic acid. 7, Caffeic acid + esculetin. 8, Syringic acid. 9, *p*-vanillin + epicatechin. 10, *cis-p*-Coumaric acid. 11, *Trans-p*-Coumaric acid. 12, Syringaldehyde. 13, *cis*-Ferulic acid + tryptophol. 14, *trans*-Ferulic acid. 15, Cinnamic acid–tartaric acid esters.

proportions of these two substances and for *cis*-ferulic acid alone, a correction factor was calculated based on the ratio of the areas under the curves at 280 and 340 nm. This correction factor was applied in calculations carried out for subsequent samples, such that the area at 280 nm, less the area at 340 nm multiplied by the factor, gave the corresponding area for tryptophol. The results obtained indicated 11·26 and 4·90 mg/litre in the white wines made from Airén and Viura grapes, respectively, 24·77 mg/litre for the red wine made from the Tempranillo grapes and 11·20 mg/litre for the red wine made from the Garnacha grapes.

Because of the broad range of mean concentration values, statistical

TABLE 3
Statistical Indices of Tryptophol Distribution in Wines made from Airén and Viura Grapes

Statistical index	*Airén* *La Mancha region*	*Viura* *Rioja region*
n	5	6
m	11·26	4·90
σ	1·45	2·74
Σx^2	644·75	189·59
S_d	1·49	
d	6·36	
$t \times S_d$	4·84 ($p<0·01$)	
V_A	6·26	
V_F	110·47	
$F_{exp.}$	17·64	
$F_{Sned.}$	10·6 ($p<0·01$)	

analysis was performed by difference of means and Student's t-test and AOV (Tables 3 and 4). Positive results, although with varying degrees of significance, were obtained for both the red and white wines. The results indicated that the differences in tryptophol content, in the young varietal wines made from the grape varieties studied, were statistically significant under the conditions set out above. The differing degrees of

TABLE 4
Statistical Indices of Tryptophol Distribution in Wines made from Tempranillo and Garnacha Grapes

Statistical index	*Tempranillo*	*Garnacha*
n	7	10
m	24·77	11·20
σ	6·91	5·75
Σx^2	12 679·67	1 594·90
S_d	3·27	
d	13·57	
$t \times S_d$	13·31 ($p<0·001$)	
V_A	193·63	
V_F	2 136·55	
$F_{exp.}$	11·03	
$F_{Sned.}$	8·2 ($p<0·01$)	

significance, obtained by the different methods of statistical analysis employed, indicates that analysis of variance is more rigorous than is difference of means.

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REFERENCES

Carrasco de la Peña, J. L. (1982). *El método estadistico en la investigación médica*, Karpos, Madrid.

Díez, C., Gómez-Cordovés, C., Blanco, M. & G. de la Calera, J. (1980). Compuestos fenólicos de pequeño peso molecular. Su influencia en la calidad de los vinagres. *Rev. Agroquim. Tecnol. Aliment.*, **20**(2), 247–56.

Hernández, T. & Sánchez, E. (1980). La separation des phénols non flavonoides par la CLHP. *C.R. Ass. Gen. Ann. Groupe Polyphenols* (Neuchatel, Switzerland) **10**, 263–8.

Nykänen, L., Puputti, E. & Suomalainen, H. (1966). Gas chromatographic determination of tyrosol and tryptophol in wines and beers. *J. Inst. Brewing*, **72**, 24–8.

Ribéreau-Gayon, P. & Sapis, J. C. (1965). Sur la présence dans le vin de tyrosol, de tryptophol, d'alcool phényléthylique et de γ-butyrolactone, produits secondaires de la fermentation alcoolique. *C.R. Acad. Sci. Paris*, **261**, 1915.

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

Sapis, J. C. & Ribéreau-Gayon, P. (1969*a*). Etude dans les vins du tyrosol, du tryptophol, de l'alcool phényléthylique et de la γ-butyrolactone, produits secondaires de la fermentation alcoolique. I—Identifications et méthodes de dosage. *Ann. Technol. Agric.*, **18**(3), 207–19.

Sapis, J. C. & Ribéreau-Gayon, P. (1969*b*). Etude dans les vins du tyrosol, du tryptophol, de l'alcool phényléthylique et de la γ-butyrolactone, produits secondaires de la fermentation alcoolique. II—Présence et signification. *Ann. Technol. Agric.*, **18**(3), 221–9.