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A Relationship Between Triglycerides and Grape-Ripening Indices

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ABSTRACT

Two varieties of Vitis vinifera, *cv Airén and Cencibel, were studied during the latter stages of ripening, and factor analysis was applied to the lipids and traditional ripening indices.*

The results suggest differences in the metabolic usage of triglycerides depending on the molecular species. As might be expected, triglycerides were used as energy sources, but the metabolic availability of certain molecular species was more directly related to the ripening process.

INTRODUCTION

The most commonly used ripening indices involve sugar and organic acid concentrations in the berries (Diez & Junquera, 1987). Total sugars or soluble solids and the ratio of glucose to fructose have been used as sugar-based indices (Fregoni, 1983). Total acid, malic acid and tartaric acid levels (Ruffner, 1982), pH, and various ratios between these acids have been used as indices of acidity (Diez & Junquera, 1987). The relationship between the sugar or soluble solids content and total acidity has also been employed as a ripening index for wine grapes (Ough & Alley, 1970; Flora & Lane, 1979; Du Plessis & Van Rooyen, 1982).

Ripening indices based on other biochemical compounds present in the berries have also been proposed, and these may furnish additional information complementing that provided by sugar and acidity indices (Du Plessis, 1983). Relationships based on polysaccharide concentration

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(Dubordie *et al.*, 1981), phenols (Sirota *et al.*, 1979; Fregoni, 1983), nitrogen compounds, especially the proline (Ough & Alley, 1970) and ammonia (Ough, 1969) concentrations, varietal aroma components, in particular terpenic compound concentration (Terrier *et al.*, 1972) and the linalool/geraniol ratio (Wagner *et al.*, 1977), and minerals, especially the K^+/malic acid ratio (Hale, 1977) have been proposed as indices. Moreover, the enzyme activity level has been suggested as a potentially very interesting indicator for determining optimum harvesting time (Fregoni, 1982; Hrazdina *et al.*, 1984).

In contrast, the lipids in grapes have not received attention in this respect. Previous work has focused on descriptions of the fatty acid (Higgins & Peng, 1976; Buman *et al.*, 1977; Gallander & Peng, 1980; Lavaud, 1982) and triglyceride compositions (Zeany, 1982; Barron *et al.*, 1988) of grapes. Nevertheless, certain researchers have mentioned, in work dealing with changes in fatty acids during grape ripening, the importance of long-chain unsaturated fatty acid concentration during ripening, in view of their influence on herbaceous aroma development in must and wine (Castelá *et al.*, 1985; Roufet *et al.*, 1987).

This paper presents a study of the relationship between lipids and grape-ripening indices by applying factor analysis to lipid variables and ripening variables for two varieties of *Vitis vinifera*, white Airén grapes and red Cencibel grapes. The Airén variety is the most cultivated in Spain and the Cencibel variety is one of the most important for red wine elaboration.

Based on the lipid composition of grapes reported in an earlier paper (Barron *et al.*, 1988), the lipid variables analyzed were: the major fatty acids, linoleic (L), oleic (O), stearic (S) and palmitic (P) acids; the triglyceride molecular species trilinolein (LLL), oleodilinolein (LOL), palmitodilinolein (LPL), linoleodiolein (OLO), stearodilinolein (LSL), linoleo-oleo-palmitin (LOP), and triolein (OOO), and the total lipids in the berries.

The total sugars/total acidity, tartaric acid/malic acid and glucose/fructose ratios, total sugars, berry weight, total polyphenols, and total anthocyanins (this latter for the Cencibel grape variety only) were used as the ripening variables.

MATERIALS AND METHODS

Samples

Samples were taken at two experimental vineyards growing *Vitis vinifera*, cv Airén and Cencibel, operated by the Comunidad Autónoma de Madrid. Random samples were taken from 18 vines in a 'Z' pattern three times a week

between 3 and 24 September. Four groups of 100 berries each were randomly selected from all the samples collected each day for use in the quantitative analyses.

Chemical analysis

Lipids were extracted from the frozen whole grape berries according to the method of Higgins and Peng (1976). Triglycerides were purified on a silicic acid column and analyzed by HPLC using the method described in an earlier paper (Barron *et al.*, 1987). The fatty acids in the total lipid extract were analyzed by GLC after saponification with potassium hydroxide in methanol (Barron *et al.*, 1988).

Total sugars and total acidity were assayed following the *Métodos Oficiales de Análisis* (1973). Tartaric and malic acids and the reducing sugars, glucose and fructose, were analyzed using enzymatic methods (Boehringer Mannheim, 1982). Total polyphenols were analyzed using Folin-Ciocalteus reagent (Folin & Ciocalteus, 1927), total anthocyanins by means of color changes with the pH of the medium (Paronetto, 1977).

The results of the chemical analysis were expressed as mg/100 berries.

Statistical analysis

The factor analysis was applied to the samples, without regard to grape variety and for each variety separately, in order to study the relationships between grape lipids and ripening variables.

The factor matrix was estimated from the correlation matrix by means of principal component analysis using program 4M from statistical package BMDP83 (Frane *et al.*, 1983). For ease of interpretation, factor scores were rotated using the Varimax method. Program 4M was run on a CYBER 155/855 computer (Control Data Corporation, Arden Hills, USA).

RESULTS AND DISCUSSION

Factor analysis of all samples combined, without regard to grape variety, yielded three eigenvalues greater than unity, 8·588, 4·164 and 1·958, corresponding to three factors that, together, explained 86·53% of the variance.

Table 1 presents the rotated and unrotated loadings for these factors, with rotated factor loadings less than, or equal to, an absolute value of 0·250 set to zero. The rotate factor loadings show factor 1 to be highly correlated with lipids and this was therefore defined as the energy reserve factor in the berry.

TABLE 1

Rotated and Unrotated Factor Loadings for Factors I, II, and III for Factor Analysis of the Airén and Cencibel Grape Varieties Combined

(Rotated factor loadings less than or equal to an absolute value of 0·250 set to 0)

Variable	*Unrotated factors*			*Rotated factors*		
	I	*II*	*III*	*I*	*II*	*III*
linoleic acid	0·958	0·142	−0·084	0·962	0·000	0·000
oleic acid	0·960	−0·147	−0·045	0·924	−0·300	0·000
palmitic acid	0·972	0·082	−0·095	0·972	0·000	0·000
stearic acid	0·858	0·368	−0·276	0·931	0·269	0·000
LLL	0·885	0·253	−0·254	0·941	0·000	0·000
LOL	0·948	−0·028	0·070	0·900	−0·257	0·000
LPL	0·937	0·167	−0·162	0·962	0·000	0·000
OLO	0·895	−0·113	−0·221	0·905	0·000	0·000
LOP	−0·033	0·894	−0·201	0·000	0·854	0·314
LSL	0·078	0·613	−0·386	0·000	0·692	0·000
OOO	0·452	−0·673	−0·098	0·390	−0·605	−0·386
total lipids	0·934	−0·032	0·253	0·844	−0·351	0·319
total polyphenols	0·515	−0·413	0·635	0·313	−0·777	0·371
sugar/acidity	0·242	0·696	0·627	0·000	0·000	0·930
tar/mal	−0·442	0·705	−0·292	−0·289	0·829	0·000
glucose/fructose	−0·298	−0·466	−0·714	0·000	0·000	−0·885
sugar weight	−0·118	0·936	0·256	0·000	0·673	0·705

Tar = tartaric acid, mal = malic acid, L = C18:2, O = C18:1, S = C18:0, P = C16:0.

Factors II and III were correlated with traditional ripening indices and can therefore be defined as ripening factors.

Factor II displayed high correlations with total polyphenols, and the indices of tartaric acid/malic acid and total sugar × weight, and it was defined as the ripening factor associated with the secondary metabolism. It can be regarded as the industrial ripening factor, in view of the important role of polyphenolic compounds in determining wine quality, and the importance of the hydroxy acids in the must during wine-making.

Factor III showed high correlations with the ripening indices of total sugars/total acidity, glucose/fructose and total sugars × weight, and hence it can be defined as the ripening factor related to the primary metabolism, i.e. the physiological maturity factor.

The grape samples distribution (standardized data) in the two-dimensional coordinate system defined by factors II and III showed a dependence of industrial ripening factor on grape variety, while the physiological ripening factor did not (Fig. 1).

Total lipids, fatty acids, and major triglyceride molecular species LLL,

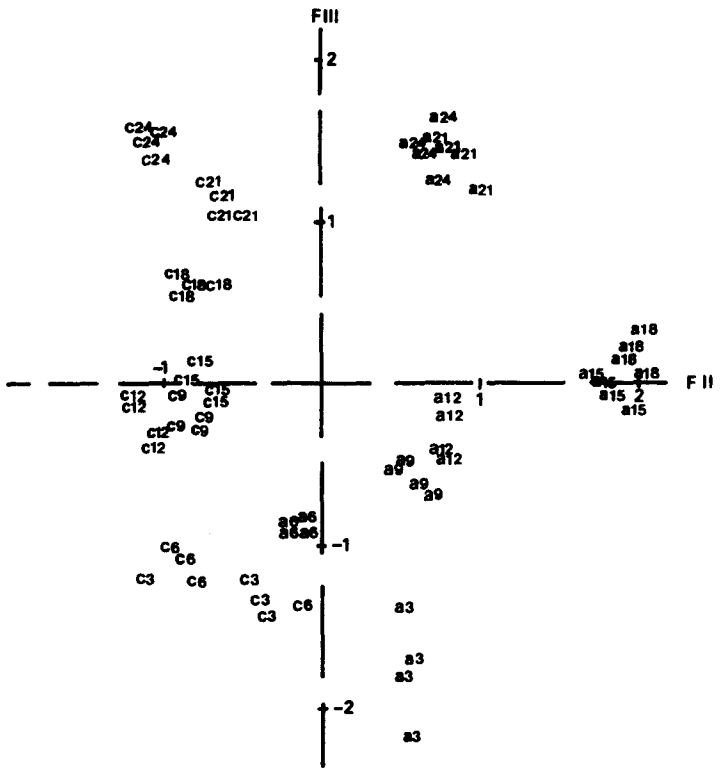

Fig. 1. Grape sample distribution (standardized data) in the two-dimensional coordinate system defined by factors II and III, selected in factor analysis of the Airén and Cencibel grape varieties combined. FII = ripening factor related to secondary metabolism, FIII = ripening factor related to primary metabolism, c_i = Cencibel grape samples, a_i = Airén grape samples, i = date.

LOL, LPL, and OLO were correlated with the energy reserve factor, while the other triglyceride molecular species LOP, LSL and OOO, were correlated with the secondary ripening factor. These results would seem to indicate differences between triglyceride molecular species in their metabolic usage during grape ripening.

As in the case set out above, factor analysis for the Airén grapes separately yielded three eigenvalues greater than unity, 10·369, 3·281, 1·921, explaining 91·59% of the variance. Table 2 shows that the meanings of the factor scores were analogous to those for the factor analysis for the two grape varieties combined. Factor 1 was the energy reserve factor, factor 2 was the ripening factor related to fruit growth, and factor 3 was the ripening factor related to the secondary metabolism.

Separate factor analysis on the Cencibel grapes yielded two eigenvalues greater than unity, 8·408 and 6·395, together explaining 82·21% of the variance. Table 3 shows factor A to be correlated with most of the lipid

TABLE 2
Rotated and Unrotated Factor Loadings for Factors 1, 2, and 3 for 3 for Factor Analysis of the Airén Grapes Separately
(Rotated factor loadings less than or equal to an absolute value of 0·250 set to 0)

Variable	*Unrotated factors*			*Rotated factors*		
	1	*2*	*3*	*1*	*2*	*3*
linoleic acid	0·962	−0·225	−0·127	0·990	0·000	0·000
oleic acid	0·967	−0·223	−0·085	0·986	0·000	0·000
palmitic acid	0·957	−0·265	−0·016	0·976	0·000	0·000
stearic acid	0·966	−0·168	−0·109	0·974	0·000	0·000
LLL	0·938	−0·254	0·023	0·947	0·000	0·000
LOL	0·911	−0·121	−0·284	0·943	0·000	0·000
LPL	0·946	−0·260	0·063	0·949	0·000	0·000
OLO	0·866	−0·222	−0·121	0·898	0·000	0·000
LOP	0·779	0·528	0·092	0·549	0·751	0·000
LSL	0·900	0·051	−0·091	0·844	0·324	0·000
OOO	0·060	−0·532	0·712	0·000	0·000	0·883
total lipids	0·961	0·008	0·066	0·881	0·382	0·000
total polyphenols	0·440	0·676	−0·421	0·289	0·515	−0·693
sugar/acidity	0·525	0·801	0·247	0·000	0·953	0·000
tar/mal	0·321	−0·427	0·726	0·286	0·000	0·849
glucose/fructose	−0·300	−0·641	−0·653	0·000	−0·928	−0·253
sugar weight	0·541	0·794	0·256	0·000	0·957	0·000

Tar = tartaric acid, mal = malic acid, L = C18:2, O = C18:1, S = C18:0, P = C16:0.

variables, thus corresponding to the energy reserve factor, while factor B was, in this instance, correlated with all the ripening variables. The existence of a single ripening factor indicates that, in the red Cencibel variety, the processes related with the secondary metabolism, chiefly the synthesis of anthocyanins and other phenolic compounds, play a greater role in determining the ripening stage than they do in the white Airén variety.

Comparing the distribution of lipid variables among the factors for each of the two grape varieties (Tables 2 and 3), total lipids, and the individual fatty acids can be seen to contribute to the energy reserve factor. In contrast, the triglycerides were shared between the energy reserve factor and the factors associated with grape ripening. For the Cencibel grapes, triglycerides OOO and LOP were significantly correlated with the ripening factor (factor B) while, for Airén grapes, these same two molecular species were correlated with different ripening factors, LOP with the factor for fruit growth (factor 2) and OOO with the factor for the secondary metabolism (factor 3).

These results would seem to indicate that molecular species LOP and OOO might act as more readily available energy sources for the metabolic

TABLE 3
Rotated and Unrotated Factor Loadings for Factors A and B for Factor Analysis of the Cencibel Grapes Separately
(Rotated factor loadings less than or equal to an absolute value of 0·250 set to 0)

Variable	*Unrotated factors*		*Rotated factors*	
	A	*B*	*A*	*B*
linoleic acid	0·972	−0·003	0·958	0·000
oleic acid	0·912	−0·324	0·955	0·000
palmitic acid	0·957	−0·087	0·957	0·000
stearic acid	0·963	−0·178	0·979	0·000
LLL	0·902	−0·229	0·928	0·000
LOL	0·957	0·063	0·931	0·000
LPL	0·927	−0·135	0·937	0·000
OLO	0·677	−0·618	0·744	−0·491
LOP	0·512	−0·639	0·615	−0·540
LSL	−0·332	−0·062	−0·317	0·000
OOO	−0·154	−0·883	0·000	−0·897
total lipids	0·862	0·285	0·800	0·430
total polyphenols	−0·089	0·935	−0·250	0·906
total anthocyanins	0·017	0·934	0·000	0·923
sugar/acidity	0·362	0·893	0·000	0·942
tar/mal	0·226	0·799	0·000	0·826
glucose/fructose	−0·485	−0·734	−0·351	−0·807
sugar weight	0·419	0·895	0·257	0·954

Tar = tartaric acid, mal = malic acid, L = C18:2, O = C18:1, S = C18:0, P = C16:0.

changes taking place during the latter stages of grape ripening than the other triglycerides analyzed.

In the case of Cencibel grapes, the triglyceride LSL was not significantly correlated with either of the two factors selected (Table 3), while in the Airén grapes, this triglyceride was correlated with the energy reserve factor (Table 2). These results may explain why in the factor analysis for both grape varieties combined, triglyceride LSL was anomalously correlated with ripening factor II (Table 1).

CONCLUSIONS

It would appear that the metabolic role of the triglycerides was dependent upon each molecular species involved, and differences were recorded between the two grape varieties studied. LOP and OOO triglyceride molecular species exhibited metabolic availability that seemed to be more directly related to the metabolic changes taking place during ripening,

whereas the others appeared to act as energy storehouses. Particularly, OOO molecular species might be related to the secondary metabolism of grapes, chiefly the synthesis of phenolic compounds.

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