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# TRIACYLGLYCEROL CHANGES IN GRAPES IN LATE STAGES OF RIPENING

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**Key Word Index**—*Vitis vinifera*; Vitidaceae; ripening; grapes; triacylglycerols; regression analysis.

**Abstract**—Triacylglycerol composition was studied in fruits of Airen and Cencibel grape varieties (*Vitis vinifera*) in late stages of ripening. The lipids showed an alternating pattern, reaching a maximum concentration during the final part of the ripening period studied. Their evolution was similar in the two varieties, reaching physiological maturity a few days earlier in the Airen grapes. Moreover, differences were observed in some of the molecular species between the two grape varieties and within them. A polynomial regression model was useful for explaining the evolution of triacylglycerols during maturation of the two grape varieties studied.

## INTRODUCTION

Fruits undergo variations in fatty acid content during growth, ripening and senescence [1]. There are few papers dealing with fatty acids in grapes, however, although interest in this aspect has increased of late, since fatty acids have been shown to be involved in the formation of the volatile compounds responsible for the herbaceous aroma of some wines [2–4]. Some authors have noted that the total lipid content in the berry rises towards the end of ripening, with the ratio of neutral lipids to polar lipids also tending to increase [5–7]. The total lipid content of the berry reaches its highest point in véraison, chiefly as a result of an increase in neutral lipids taking place up until that time, and afterwards the amounts of neutral lipids fall sharply, such that by the end of ripening the lipid content is slightly higher than that during the unripe stage [5, 7]. These observations suggest that certain lipids might be used as an energy source during growth and ripening of grapes.

There would appear to be some disagreement among different workers about fatty acid behaviour during the vegetative cycle of grapes. Roufet *et al.* [8] reported essentially no variation in saturated fatty acid and linoleic acid concentrations in unripe and ripe grapes, whereas the linolenic acid content declined appreciably and the oleic acid content underwent a slight increase. On the other hand, Castelá *et al.* [7] found that linoleic and palmitic acid in Macabeo grapes decreased from the unripe stage to the onset of ripening, remaining constant at the time around maturity. They also noted that the ratio of unsaturated to saturated acids tended to decrease, attaining a value of 8.4 at maturity.

Some work on fruit ripening has indicated that most of the compounds present undergo fluctuations, with a minimum and a maximum concentration [9–12]. Simple linear regression models do not readily explain such fluctuations. Maujean *et al.* [12] reported that the total sugars in grapes followed an exponential pattern during ripening. Reports about variations in molecular species of triacylglycerols (TG) during grape ripening are not found in the literature. Belenko *et al.* [13] recorded an increase in total TG in grape seeds during ripening and variations in their fatty acid composition.

The present paper describes changes in the concentrations of individual TG molecular species in Airen and Cencibel grapes in the late stages of ripening. Airen is the most commonly cultivated variety in Spain whereas the Cencibel variety is one of the most important for the manufacture of red wine. Polynomial regression was applied to develop a mathematical model to explain the experimental data.

## RESULTS AND DISCUSSION

HPLC chromatograms of the TG from the two grape varieties studied were similar. HPLC peaks were identified as described previously [14] as LLL (peak 1), LOL (peak 2), LPL (peak 3), OLO (peak 4), LSL-LOP (peak 5), OOO (peak 6) and LOS-OPO-LSP (peak 7), where L = linoleic, O = oleic, P = palmitic and S = stearic acid. TG LLL, LOL, LPL and OLO were quantified by HPLC. Molecular species LSL and LOP, not resolved by HPLC (peak 5), were quantified by means of GC analysis of the fatty acids S and P, respectively, after collecting the fraction at the outlet of the HPLC equipment. In the same way OOO was quantified by means of GC analysis of the fatty acid O, because the area of the HPLC peak 6 was difficult to measure. TG LOS, OPO and LSP, not resolved by HPLC (peak 7), could not be quantified by GC since their fatty acids constituents L, O, S and P occurred in more than one molecular species.

Coefficients of variation of less than 4% were obtained for all TG and fatty acids after performing five replications of the quantitative analysis on one of the grape samples of each variety. Quantitative results corresponding to four groups of 100 berries of each sample collection provided coefficients of variation minus or equals to 10%. Therefore, the experimental data presented reasonable dispersion values. Application of polynomial regression to the TG and fatty acid results yielded sixth- or seventh-degree polynomials, with this degree representing practical limit values, i.e. the highest possible degrees for the

polynomials in the present instance. Variations in the TG LSL in Airen grapes were described by a fourth-degree polynomial. The regression coefficients estimated were in all cases statistically non-zero at $p > 0.05$, and the residuals were randomly distributed around the mean value 0 and displayed a normal probability distribution. A polynomial regression model was therefore suitable for explaining the evolution of TG and fatty acids during grape ripening.

The variations in unsaturated (UFA) and saturated (SFA) fatty acids from the TG fraction for each grape variety are presented in Fig. 1. The behaviour of the UFA, the major component in the grapes [7, 14], was similar in the two varieties, presenting an alternating pattern reaching a maximum during the final part of the ripening period studied. The maximum was attained *ca* four days earlier in the Airen variety. This may be because Airen grapes reach physiological maturity sooner than Cencibel grapes. The concentration of SFA, the minor component in grapes [6, 7, 14], varied little during ripening in the two varieties studied. On the other hand, the mean total fatty acid content was slightly higher in Cencibel grapes (940 mg/100 berries) than in Airen grapes (647 mg/100 berries), chiefly because of the UFA contribution. The UFA/SFA ratio had values of *ca* 8 for Airen and 9 for Cencibel grapes. These values are similar to those reported by Castelá *et al.* [7] for Macabeo grapes.

The amounts of total TG in the grapes as quantified by weight are shown in Fig. 2. Triglyceride variation was similar to that of the fatty acids (Fig. 1) in both grape varieties. The time difference in ripening in the Airen and Cencibel varieties is again evident. The fluctuation range, given as the difference between the maximum and minimum values expressed as a percentage, was lower in Cencibel (*ca* 50%) than that in Airen (*ca* 80%) grapes. This suggests that the TG, which are primarily energy repositories, were used more continuously in Cencibel than in Airen grapes, in as much as energy requirements may be higher in red grape (Cencibel) varieties than in white grape (Airen) varieties, due to the higher secondary metabolic activity in the berry, chiefly in the synthesis of phenolic compounds during ripening.

The concentrations of the TG of the LLL-OOO transesterification series are presented in Fig. 3. In both grape varieties, as more positions of the glycerol molecule were esterified by oleic acid, differences in content arise during the period spanning the minimum and final maximum (OLO) concentrations, such that the behaviour was completely different from that of the others when all three positions were esterified by this acid (OOO). This may mean that OOO plays a metabolic role during ripening distinct from that of the other TG in the series.

The concentration pattern for the TGs LPL, LSL and LOP is shown in Fig. 4. LPL behaved like LLL and LOL (Fig. 3) in both varieties, and these three species are the major components in grapes [14, 20]. In Cencibel grapes, the changes in the TG LSL and LOP were similar to that of OLO (Fig. 3), although the former showed a more downward trend. In Airen grapes, LSL displayed no appreciable differences with respect to the major TG components, although LOP showed an intermediate peak, like OLO (Fig. 3) and followed a more upward trend.

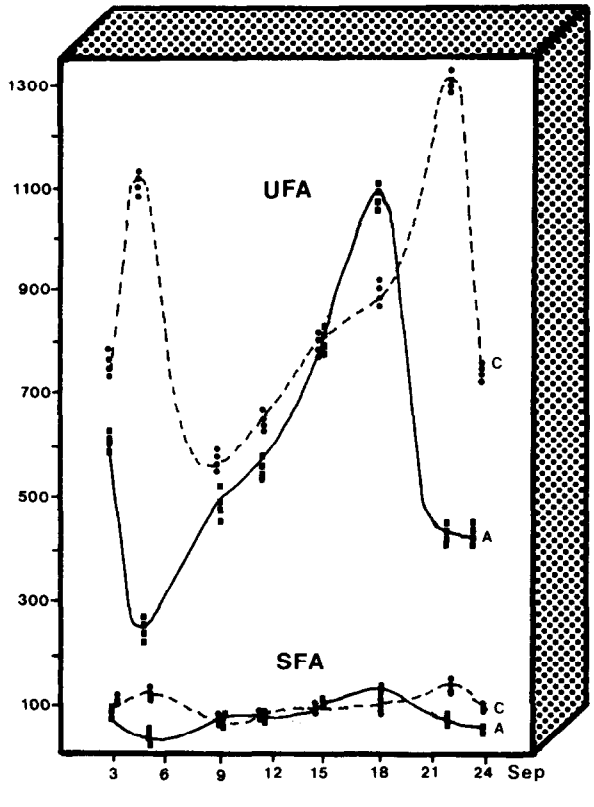


Fig. 1. Changes in amounts (mg/100 berries) of unsaturated (UFA) and saturated (SFA) fatty acids in Airen (A) and Cencibel (C) grape varieties during late stages of ripening.

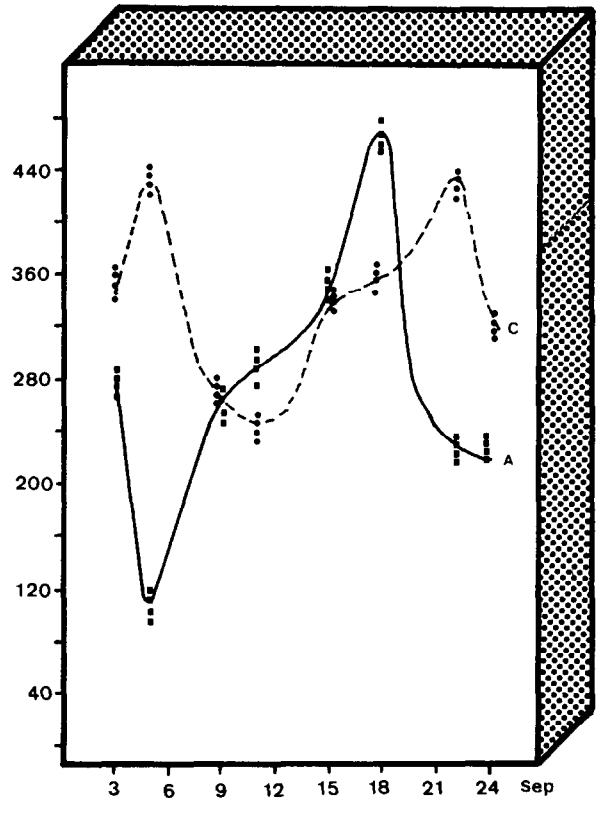


Fig. 2. Changes in amounts (mg/100 berries) of TG in Airen (A) and Cencibel (C) grape varieties during late stages of ripening.

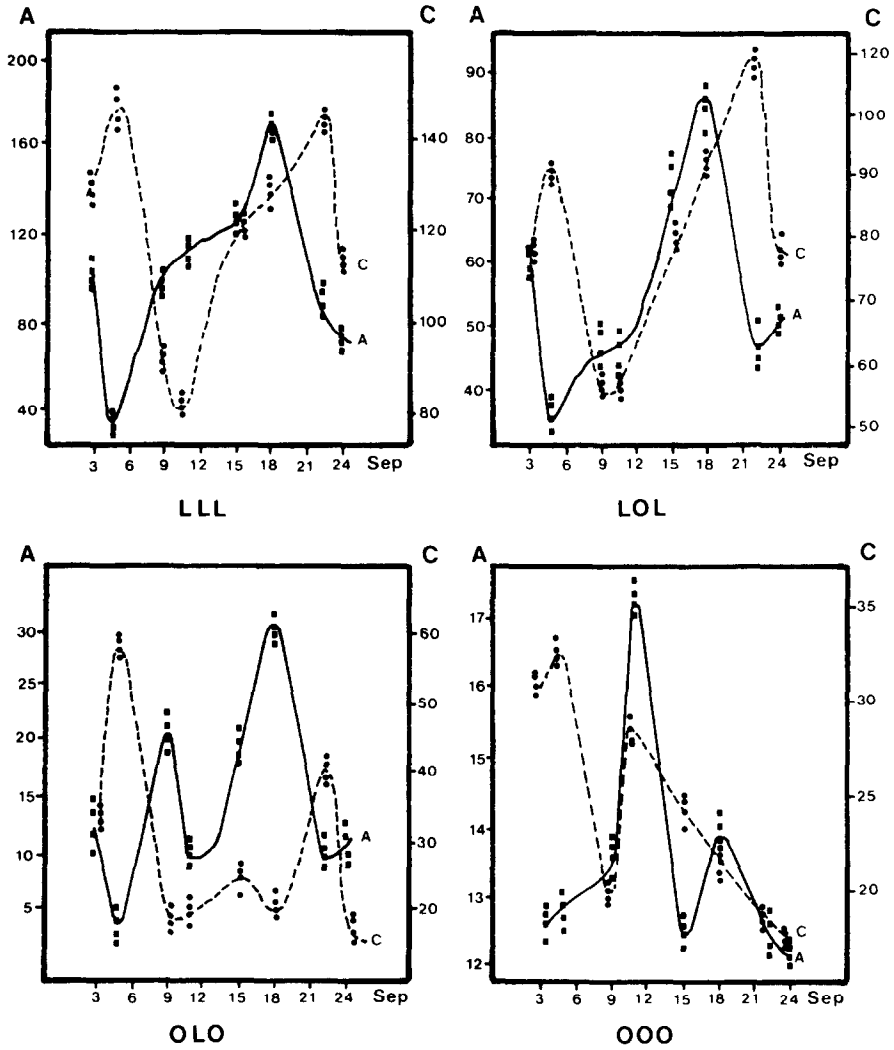


Fig. 3. Changes in amounts (mg/100 berries) of TG of the LLL-OOO transesterification series in Airen (A) and Cencibel (C) grape varieties during late stages of ripening. L = C18:2, O = C18:1.

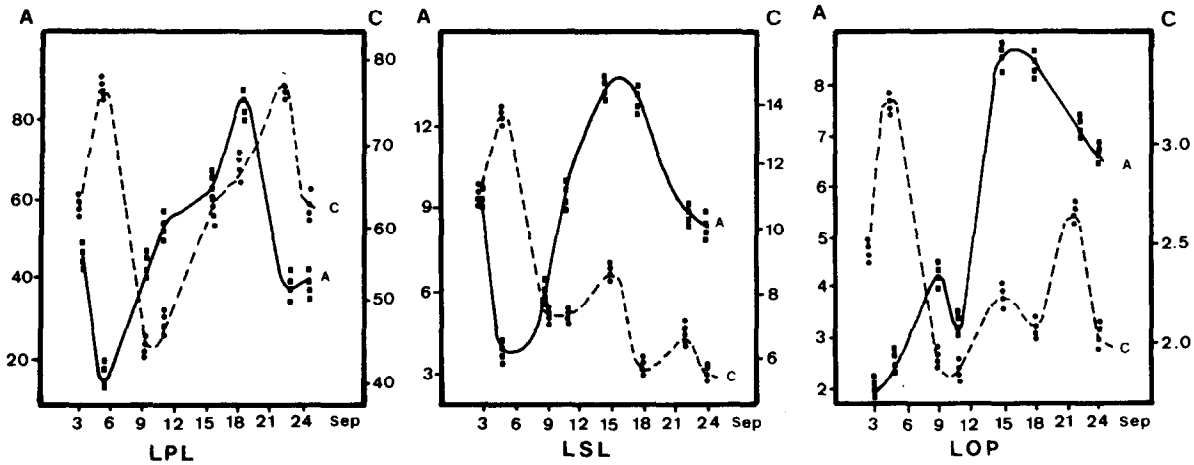


Fig. 4. Changes in amounts (mg/100 berries) of LPL, LSL and LOP TG in Airen (A) and Cencibel (C) grape varieties during late stages of ripening. L = C18:2, O = C18:1, S = C18:0, P = C16:0.

From the above there would appear to be differences between TG molecular species in their metabolic usage during grape ripening. The species OOO, LSL and LOP displayed considerable differences in comparison to the major TG. Moreover, there would appear to be differences in the two grape varieties in the metabolic usage of the species OOO, LSL and LOP.

In order to study the relationships between grape TG and physiological maturity, factor analysis was applied to the lipid variables studied, including the weight of 100 berries (WTB) as an indicator of grape ripening or growth. Factor analysis of all the samples irrespective of grape variety yielded only two eigen values higher than unity, 6.657 and 2.782, and consequently just two factors were selected, explaining 85.81% of the variance. The coefficients for these two factors, unrotated and rotated, with coefficients less than 0.250 (absolute value) for the rotated factors given as 0 are summarized in Table 1. The TG were divided between these two factors. Factor I presented higher correlations with lipids other than LSL, LOP and OOO. Factor II essentially comprised the WTB and the TG LOP, OOO and LSL. Because of the weight, this factor can be regarded as the factor of growth or ripening, whereas factor I is the factor for the energy reserves of the fruit. Consequently, the metabolic usage of TG can be inferred to differ depending on molecular species. Thus, the major TG components in grapes, LLL, LOL, LPL and OLO, serve chiefly as energy storehouses, while LSL, LOP and OOO are more related to physiological maturity. These minor TG components could be kept as a more readily available source of energy required by the metabolic processes taking place during grape ripening.

## EXPERIMENTAL

*Plant material.* Samples were collected from two experimental vineyards of *V. vinifera*, one each of the varieties Airen and Cencibel, run by the Comunidad Autónoma de Madrid. Samples were taken from 18 vines using a 'Z' pattern designed to prevent border and centre effects. Sample collection was random, with the bunch taken from each vine selected by lot from among the four cardinal points of the compass. It was carried out on eight days between 3 and 24 September. Four groups of 100 berries each were chosen at random from all the berries taken on a given day for quantitative analysis. The sample size was 32 in each grape variety.

Table 1. Coefficients for unrotated and rotated factors I and II for factor analysis of all the samples during grape ripening; coefficients for rotated factors with an absolute value of less than 0.250 have been set at 0

| Variable | Unrotated factors I | Unrotated factors II | Rotated factors I | Rotated factors II |
|---|---|---|---|---|
| LLL | 0.930 | 0.230 | 0.957 | 0.000 |
| LOL | 0.924 | −0.092 | 0.909 | 0.000 |
| LPL | 0.967 | 0.124 | 0.974 | 0.000 |
| OLO | 0.910 | −0.128 | 0.891 | 0.000 |
| LSL | 0.157 | 0.725 | 0.000 | 0.704 |
| LOP | 0.026 | 0.912 | 0.000 | 0.990 |
| OOO | 0.445 | −0.696 | 0.368 | −0.740 |
| TTG | 0.981 | 0.154 | 0.992 | 0.000 |
| UFA | 0.966 | 0.026 | 0.964 | 0.000 |
| SFA | 0.969 | 0.153 | 0.980 | 0.000 |
| WTB | −0.321 | 0.893 | 0.000 | 0.922 |

TTG = total TG; UFA = unsaturated fatty acids; SFA = saturated fatty acids; WTB = wt of 100 berries; L = C18:2; O = C18:1; S = C18:0; P = C16:0.

*Extraction of lipids.* Lipids were extracted wet from previously frozen whole grape berries using *n*-hexane–*iso*-PrOH (3:2). TG were purified by means of silicic acid CC [14]. The TG fr. was concd to dryness, weighed and redissolved in $CHCl_3$. All operations were carried out in a $N_2$ atmosphere.

*HPLC analysis.* TG analysis was carried out according to the method described in an earlier paper [15], using a Spherisorb ODS (Sugelabor) 5$\mu$ (200 × 4.6 mm) column operated at 30° and equipped with a differential refractometric detector. Molecular species of TG were identified from chromatographic data following the method described in ref. [14]. TG were quantified using peak area and expressed as mg of trilinolein (LLL)/100 berries. LLL was chosen as the ext. std because it was the major TG in the grapes. LLL solns ranging between 1.25 and 5 mg/ml were used for calibration. Quantification errors resulting from the use of an ext. std were not high, because natural TG mixts exhibit very similar responses in the RI detector [16]. Reproducibility of the HPLC method was evaluated by performing five replications on one of the grape samples.

*GC analysis.* Me esters of fatty acids (FA) from the total lipid ext. were saponified with KOH and prepd according to the method of ref. [17]. GC analysis was carried out as described in ref. [14] using a Carbowax 20 M SP 1000 liquid phase capillary column (20 m × 0.20 mm). The amounts of each individual species for those TG not quantifiable by HPLC were established by GC analysis of the corresponding frs collected at the outlet of the HPLC column and quantifying one of the FA present in the TG molecule. Results are expressed as mg TG/100 berries, taking into account the corresponding molar transformation [mg TG = mg FA$*M_r$ (TG)/$M_r$ (FA)]. Reproducibility of the GC method was evaluated by performing five replications on one of the grape samples.

*Statistical analysis.* Polynomial regression was applied to develop a mathematical model to explain the experimental data. Estimation of regression coefficients and residual analysis were performed using program 5R of the BMDP83 statistical package [18]. The degree of the polynomials was established by means of goodness-of-fit test (F). This is a test of the lack of the model at each degree, relative to the residual mean square from fitting the polynomial of highest degree. A high value for F is an indicator of poor fit, and more terms, or higher degree, are needed. The tail probability ($p$) was more than 0.05. Moreover, factor analysis of all the samples was performed to study the relationships between TG and physiological maturity of grapes. The factor matrix was estimated from the correlation matrix according to the method of principal components using program 4M in the BMDP83 statistical package [19]. Factors were rotated using the Varimax method to facilitate interpretation of the analysis results. Programs 5R and 4M were run with a CYBER 155/855 CDC computer.

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