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CHANGES IN GRAPE SEED, SKIN AND PULP CONDENSED TANNINS DURING BERRY RIPENING: EFFECT OF FRUIT PRUNING

ALTERAÇÕES AO LONGO DA MATURAÇÃO NOS TANINOS CONDENSADOS DA GRAINHA, PELÍCULA E POLPA: INFLUÊNCIA DA MONDA DE CACHOS

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ABSTRACT

The purpose of the present work was to study the evolution of condensed tannins according to their degree of polymerization in seeds, pulp and skins, along the maturation using Cabernet Sauvignon and Tinta Roriz (*Vitis vinifera* L.) varieties.

Additionally for this study a witness of each variety was compared with two modalities of fruit pruning, one of 50% and another one of 75%.

Quantitative changes in the condensed tannins with different degree of polymerization were measured using fractionation on C_{18} Sep-Pak cartridges followed by reaction with vanillin, and also by HPLC.

The results showed that for both two varieties and respective modalities, the concentration of condensed tannins (whatever the degree of polymerization) in any part of grape berry (pulp, seeds and skins) were gradually decreased throughout the ripening period

At harvest, several major analytical parameters of grape berries of the two varieties and for two modalities were not significantly changed. However, significant difference was observed in condensed tannin levels between the control and the pruning modalities.

Keywords: pruning, tannins, polymerization, maturation, Cabernet Sauvignon and Tinta Roriz.

Palavras-Chave: Monda, taninos, polimerização, Cabernet Sauvignon e Tinta Roriz.

INTRODUCTION

Proanthocyanidins also called condensed tannins (oligomeric and polymeric flavan-3-ols) are presented in the solid parts of the cluster (skins, seeds and stems) and also in the pulp, through in very reduced quantities (Lea *et al.* 1979 ; Czochanska *et al.* 1979 ; Bourzeix *et al*. 1986 ; Ricardo-da-Silva *et al*. 1991 a ; 1992 a,b ; Escribano-Baillon *et al*. 1992 ; Prieur *et al.* 1994 ; Jordão *et al*. 2001 a,b ; Sun *et al*. 2001 ; Kennedy *et al*. 2000 a,b ; 2001 ; Harbertson *et al*. 2002 ; Downey *et al*. 2003). They are of prime technological importance as contributors to colour, bitterness, astringency and aging behaviour of the red wine (Timberlake and Bridle 1976 ; Singleton and Noble 1980 ; Haslam, 1980 ; Ribéreau-Gayon *et al.* 1983 ; Ricardo-da-Silva *et al.* 1991 c ; Sarni-Machado *et al*. 1996 ; Gawel, 1998). In the last years condensed tannins have also been motive for study, as far as their potential beneficial proprieties in the domain of human health are concerned (Masquelier, 1988 ; Ricardo-da-Silva *et al.* 1991 b ; Kanner *et al.* 1994 ; Teissedre *et al.* 1996 ; Landrault *et al*. 2003).

In the case of *Vitis vinifera* grapes and of the resulting wines the proanthocyanidins are mainly procyanidins, i.e., oligomers and polymers of (+)-catechin and (-)- epicatechin linked by C-C bonds. Bonds C_4-C_8 can occur, and also of the type C_4-C_6, being the latter less frequent. Prodelphinidins can also appear with procyanidins, especially in grape skins and in red wines (Souquet *et al*. 1996; 2000, Sun *et al.* 1998a).

Quite often procyanidins are also found sterified with gallic acid in carbon 3.

Proanthocyanidins in seeds are formed by unities of (+)-catechin, (-)-epicatechin and (-)- epicatechin 3-O-gallate while in skins and stems can be distinguished from those in the seeds by the presence of (-)- epigallocatechin (Souquet *et al*. 2000; Crespy, 2002).

Seeds present the highest proportion of proanthocyanidins, followed in decreasing order by stems, skins and pulp, in the latter in very reduced quantities (Bourzeix *et al.* 1986 ; Ricardo-da-Silva *et al.* 1992 a ; Hmamouchi *et al*. 1994 ; De Freitas *et al*. 2000 ; Jordão *et al*. 2001 b ; Sun *et al.* 2001 ; Downey *et al*. 2003). However skin tannins have a higher degree of polymerazation than those contained in the seeds or in the stems (Prieur *et al*. 1994 ; Labarbe *et al*. 1999 ; Souquet *et al*. 1996; 2000).

Proanthocyanidins present in the wine and in the grape are found in highly polymerized forms (Czochanska *et al.* 1979 ; Sun *et al*.1998 a, 1999). The quantitative analysis and the distribution of those polymerized forms is of special relevance since chemical proprieties of proanthocyanidins depend, largely, on the degree of polymerization (Spranger *et al.* 2000).

In the last 30 years, several authors studied the evolution of catechins and small oligomeric procyanidins in skins, seeds, pulp and stems along maturation (Dumarzet *et al*. 1973 ; Czochanska *et al.* 1979 ; Romeyer *et al*. 1986 ; Fernandez-de-Simon *et al*. 1992 ; De Freitas and Glories 1999 ; De Freitas *et al*. 2000 ; Jordão *et al.* 1998 ; 2001 a,b ; Mateus *et al.* 2001), but few works presented the evolution during grape cluster ripening for the higher polymerized tannins. Concerning these large molecules, several authors presented results about proanthocyanidins evolution in skins and seeds during maturation using an acid-catalyzed cleavage of the compounds, in the presence of phloroglucinol or toluene-á-thiol (Kennedy *et al*. 2000 a,b ; 2001 ; 2002 ; Harbertson *et al.* 2002 ; Downey *et al*. 2003). Thus, the purpose of this work was to study the evolution of all condensed tannins, from the veraison and up to enological maturation, according to their degree of polymerization in the different parts of the berry (pulp, seeds and skins), performing an alternative methodology based on a fractionation of berry flavanols in C_{18} Sep-Pak cartridges, followed by their quantification with the vanillin assay. This methodology was already used by Jordão *et al*. (2001 b), in a similar study about condensed tannins evolution, but only in bunch stems during berry maturation.

In addition, we also evaluated the effects of several levels of cluster pruning (at veraison time) in all the different parameters studied, since this viticulture practice is nowadays associated with an increase in the quality of wines.

MATERIALS AND METHODS

Grape samples

Prunning experiments were made in Quinta de Pancas, in the Portuguese region of Estremadura.

The studied varieties were Cabernet Sauvignon and Tinta Roriz (also knew as Tempranillo and Aragonez) (*Vitis vinifera*, L).

To the experiments 100 vines of each variety were used without cluster pruning that served as control. The terminology used for the control of Cabernet Sauvignon was C12 and for the control of Tinta Roriz was R12. In the second experiment, 100 vines of each variety were used but with a cluster pruning so as to get a production of 50% in respect to the witness (control). In this case, the terminology used in the case of Cabernet Sauvignon was C6 and in the case of Tinta Roriz R6. Finally, in the third modality, 100 vines of each variety were used with a cluster pruning of 75% smaller in respect to the control, in this case the terminology used was C2 for Cabernet Sauvignon and R2 for Tinta Roriz.

For both varieties, the veraison took place on 9/08/2002, and fruit pruning on 13/08/2002. The grapes of these varieties were harvested on 1/10/2002 in the case of Cabernet Sauvignon and on 23/09/2002 in the case of Tinta Roriz.

The soil was argillaceous and the plantation beat was 2,5x1m for both varieties. The root-stock used was the SO4 for Cabernet and the 41B for Tinta Roriz.

To estimate the production per hectare the following calculation was made:

Number of clusters per vine x number of vines per hectare x medium weight of clusters.

It must be referred that this calculation took account the number of fails per hectare. The production estimation (t/ha) was 7,7 ; 3,8 ; 1,9 respectively for C12 ; C6 ; C2 and 11,6 ; 5,8 ; 2,9 for R12 ; R6 and R2.

For each modality the picking of 200 berries was made in duplicate according to the proposal of Carbonneau *et al*. (1991), having the berries been carried afterwards to the laboratory where they were frozen at the temperature of –18ºC, until the analysis was made. In spite of the possibility that this deep freezing and thawing can lead to tannins migrations among the various components of the berry, it had to be done since the method used for fractionation and determination (Sun *et al.* 1998 a,b) of flavanols according to their degree of polymerisation is a very long and complex one, making it impossible to execute it on the same day when the samples were picked.

Physicochemical analysis of grapes

The following physiochemical parameters of grapes were analyzed using the methods recommend for OIV (1990): weight of 200 berries, potential alcohol degree, pH, titrable acidity, total phenols, total anthocyanins, color density, color hue, malic acid, tartaric acid and residual sugars.

Extraction of phenolic compounds from solid parts of the grape

This extraction followed the methodology proposed by Bourzeix *et al*. (1986).

The berries in the course of defrosting were separated in their components: seeds, pulp and skins. The seeds were smashed with the help of a coffee-bean miller. Afterwards each component was weighted, put into pure methanol, and subject to a nitrogen atmosphere during 1 minute. With the purpose to avoid any possible oxidation of the samples, 1g/l of ascorbic acid was added. Each sample was subject during 16 hours to a temperature of –24º, after this period the samples of the berry compounds suffered 4 successive macerations:

- Methanol/distilled water (80/20 v:v) at room temperature during 4 hours

- Methanol/distilled water (50/50 v:v) at room temperature during 4 hours

- Distilled water at –24ºC during 15 hours

- Acetone/distilled water (75/25 v:v) at room temperature during 1 hour

After each maceration the extracts were drained and mixed.

Fractionation of flavanols according to their degree of polymerization by C_{18} Sep-Pack cartridges.

According to Sun *et al.* (1998 a,b) flavanols (proanthocyanidins or condensed tannins) can be separated in monomers (catechins), oligomers (polymerization degree around 2 to 12-15) and polymers (polymerization degree, in general > 12-15).

After the extraction of phenolic compounds from the solid parts of the grape, they were fractionated by Waters C_{18} Sep-Pak cartridges (Waters, Milford M.A) followed the methodology purposed by Sun *et al*. (1998 b). Five milliliters (5 ml) of skins and pulp extracts and 2 ml for seeds were concentrated at low pressure to dryness using a rotary evaporator at <30ºC. The residue was dissolved in 20 ml of phosphate buffer pH 7,0. The pH values were leveled to 7,0 with NaOH and HCl when necessary. Two cartridges were connected in series, after this with the help of a vacuum ramp, the cartridges were conditioned with methanol (10 ml), distilled water (2x 10ml) and phosphate buffer pH 7,0 (10-15 ml). The extracts were then passed through the cartridges with a flow around 2 ml/min. Phosphate buffer pH 7,0 was added in order to eliminate the phenolic acids. The cartridges were dried (>1h.) with N_2. Ethyl acetate (25 ml) was used in the elution of monomeric and oligomeric flavan-3-ols (F_{1+2}), the polymeric proanthocyanidins fraction (F_3) was eluded by methanol (10-15 ml). F_{1+2} was taken to dryness under vacuum, redissolved in 3 ml of phosphate buffer pH 7,0 and finally added in the same series of cartridges preconditioned as described above. The cartridges were dried again with N_2 (>1h.). By sequential elution monomeric fraction (F_1) was separated from oligomeric fraction (F_2), using respectively diethyl ether (25 ml) and methanol (10-15 ml). The three fractions (F_1, F_2 and F_3) were taken to dryness under vacuum and redissolved in 3-5 ml of methanol. Sample fractionation was performed in duplicate. The total flavan-3-ols of each fraction was determined by vanillin assay as described (Sun *et al.*, 1998b).

RESULTS AND DISCUSSION

Physicochemical composition:

In Tab. I, the physicochemical composition of the modalities of cvs Cabernet

Table I

Physicochemical composition of Cabernet Sauvignon (1 Oct. 2002) (**C12:** witness modality, **C6:** 50% pruning modality, **C2:** 75% pruning modality) and Tinta Roriz (23 Sept. 2002) (**R12:** witness modality, **R6:** 50% pruning modality, **R2:** 75% pruning modality) varieties at harvest.

*Composição Físico-Quimica à vindima do Cabernet Sauvignon (1 Out. 2002) (**C12**: modalidade testemunha, **C6**: modalidade com 50 % de monda, **C2**: modalidade com 75 % de monda) e da casta Tinta Roriz (23 Set. 2002) (**R12**: modalidade testemunha, **R6**: modalidade com 50 % de monda, **R2**: modalidade com 75 % de monda).*

Modalities	C12	C6	C2	R12	R6	R2
Berry weight (200 berries) (g)	326,1	308,2	319,0	630,2	648,4	619,2
Estimated alcoholic degree (% v/v)	12,2	12,3	12,2	11,8	11,8	12,5
pH	3,46	3,48	3,51	3,54	3,54	3,60
Titratable acidity (g/l tar. ac.)	6,00	6,00	5,80	5,55	5,70	5,85
Malic acid (g/L)	1,6	1,6	1,6	3,1	2,9	2,9
Tartaric acid (g/L)	4,0	3,6	3,2	3,1	3,5	3,6
Total anthocyanins (mg/g grape)	1,6	1,3	1,6	1,7	1,7	1,8
Total phenols (o.d. x 100)	73,5	78,0	82,0	88,9	89,7	87,3
Color density (abs.u.)*	2,31	2,80	2,80	2,07	2,10	2,26
Color hue (abs.u.)*	0,512	0,520	0,508	0,603	0,619	0,594
Sugars (g/L)	368,0	344,0	368,0	352,0	348,0	360,0

Each value represents the mean from duplicate samples.
* Referred to a 1cm optical distance.

Cada valor representa a média de duas amostras.
*Referente a 1cm de distância óptica.

Sauvignon and Tinta Roriz grapes at harvest is presented. No significant differences was found in berry composition (berry weight, estimated alcoholic degree, pH, titratable acidity, malic acid, tartaric acid, total anthocyanins, total phenols, color density, color hue and sugars) among the 2 modalities and control of both varieties. These results confirm recent investigations of Queiroz *et al*. (2003) analyzing the effects of butch thinning and pruning on yield and quality of *Vitis vinifera* Tinta Roriz. However an increased of 0,7% on estimated alcoholic degree was observed in the higher pruning modality of Tinta Roriz (R2). At harvest a slight decrease in Tartaric acid and a increase of total phenols was observed for Cabernet Sauvignon with the increase of the cluster pruning level. Fig.1 shows that in what concerns total anthocyanins and total phenols a maximum of their concentration was observed for the two varieties and respective modalities after the maximum level of sugars has been reached (quantified by the % of alcoholic degree).

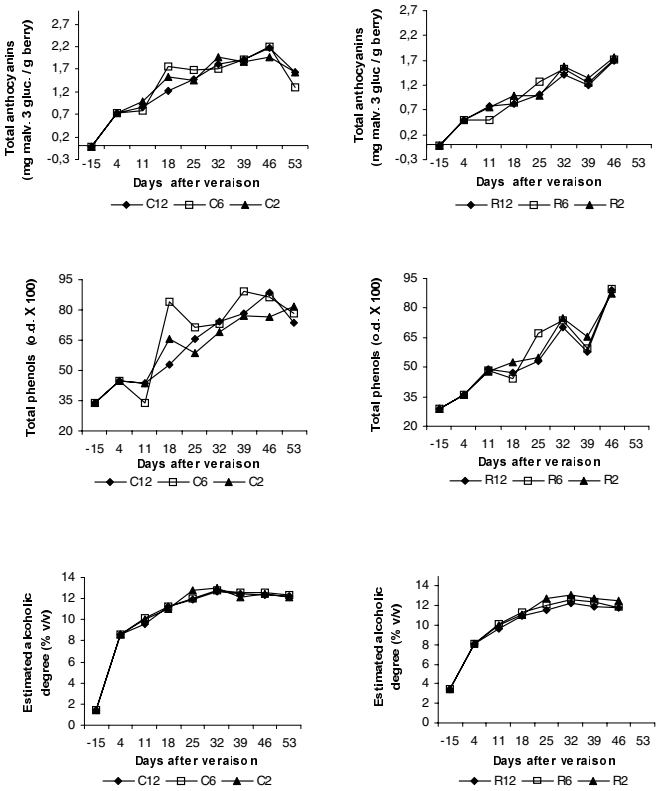

Figure 1. - Total anthocyanins, total phenols and estimated alcohol degree of red cvs Cabernet Sauvignon (**C12:** witness modality, **C6:** 50% pruning modality, **C2:** 75% pruning modality) and Tinta Roriz (**R12:** witness modality, **R6:** 50% pruning modality, **R2:** 75% pruning modality) along berry ripening.

*Evolução ao longo da maturação das antocianinas totais, fenóis totais e alcóol provável da casta Cabernet Sauvignon (**C12**: modalidade testemunha, **C6**: modalidade com 50 % de monda, **C2**: modalidade com 75 % de monda) e da Tinta Roriz (**R12**: modalidade testemunha, **R6**: modalidade com 50 % de monda, **R2**: modalidade com 75 % de monda).*

Evolution along ripening of flavanols according to their degree of polymerization: Proanthocyanidins from seeds, skins and pulp of Cabernet Sauvignon and Tinta Roriz harvested in 2002 were separated into monomers (catechins), oligomers and polymers and then quantified during grape development.

Monomeric fraction

For Cabernet Sauvignon seeds, and in the case of modality C12, a marked decrease in the first weeks was observed, followed by a stabilization in the

last weeks of ripening. The monomeric concentration in modality C6 also decreased along the ripening though with a slight increase in the two last weeks. Modality C2 was characterized by a decrease that remained invariable along the time. For the skins, in C12 modality a decrease without fluctuation along all the ripening was observed, in modalities C6 and C2 variations along the time were observed, though modality C2 presented a lower concentration than C6, except for the sample picked at harvest. For the pulp, the concentration of monomeric units was very reduced along all the ripening in all modalities, a pronounced decrease in the first 25 days after veraison was observed in all modalities, followed by a period of stable concentration. In the last weeks, the modality C2 presented a strong increase in monomeric fraction, the modality C6 remained constant and C12 suffered a strong decrease (Fig. 2a).

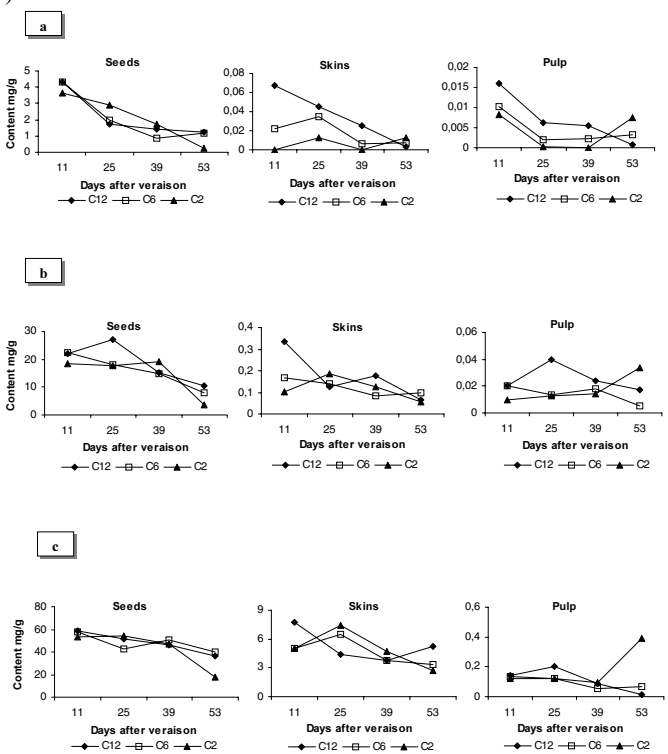

Figure 2 - Evolution of monomeric **(a),** oligomeric **(b)** and polymeric **(c)** tannins, in seeds, skins and pulp during berry ripening of the red cv. Cabernet Sauvignon (**C12**: witness modality, **C6**: 50 % pruning modality, **C2** 75 % pruning modality).

*Evolução ao longo da maturação da fracção monomérica **(a)**, oligomérica **(b)** e polimérica **(c)** na grainha, película e polpa da casta Cabernet Sauvigon (**C12**: modalidade testemunha, **C6**: modalidade com 50 % de monda, **C2**: modalidade com 75 % de monda).*

For Tinta Roriz variety, we observed that R12 and R6 modalities suffered a decrease throughout the ripening period, in what concerns the monomeric flavanol in seeds (Fig. 3a). However for R2 modality it was observed that in the two last weeks of ripening there was a slight increase of this fraction. About Tinta Roriz skins, we observed for R12 modality a decrease along the ripening until the last two weeks, and from then onwards there was a stabilization of this fraction. In modalities R6 and R2 there were fluctuations along all the ripening, having presented a decrease in the last two weeks. For the pulp, R6 and R2 modalities presented a peak 18 days after veraison, decreasing afterwards, in the case of R6 until the end of ripening, R2 presented a slight increase in the final period and R12 remained practically stable along ripening (Fig 3a).

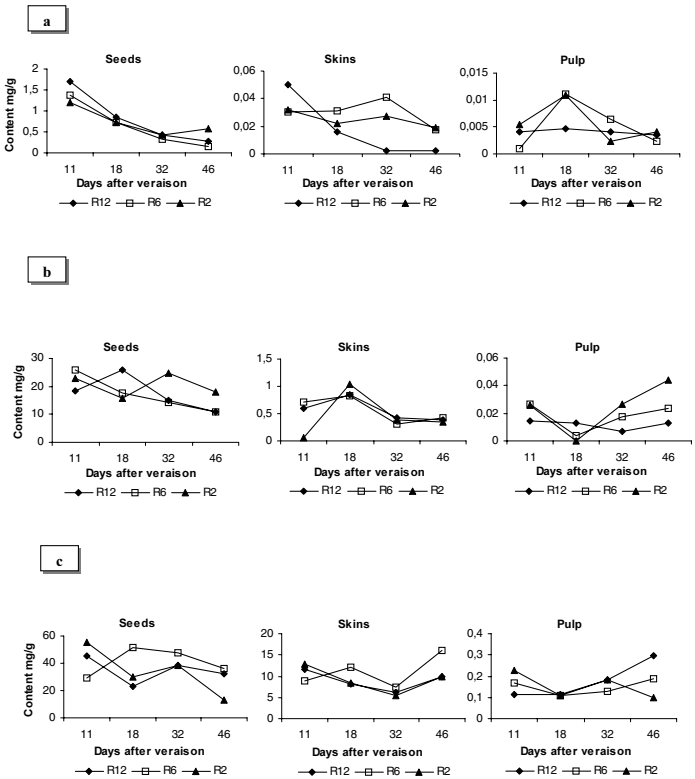

Figure 3 - Evolution of monomeric **(a),** oligomeric **(b)** and polymeric **(c)** tannins, in seeds, skins and pulp during berry ripening of the red cv. Tinta Roriz (**R12**: witness modality, **R6**: 50 % pruning modality, **R2** 75 % pruning modality).

*Evolução ao longo da maturação da fracção monomérica **(a)**, oligomérica **(b)** e polimérica **(c)** na grainha, película e polpa da casta Tinta Roriz (**R12**: modalidade testemunha, **R6**: modalidade com 50 % de monda, **R2**: modalidade com 75 % de monda).*

Oligomeric fraction

Seeds oligomeric fraction of C6 presented a decrease all along ripening, the C2 modality in the last two weeks presented a strong decrease, as for C12 a pick was found in the 25º day after veraison followed also by a strong decrease. For the skins the oligomeric fraction of Cabernet Sauvignon presented some variations along the ripening, although C6 and C2 modalities presented very similar values by the beginning and by the end of ripening. As for C12 a very pronounced decrease was observed along all ripening. For Cabernet Sauvignon pulp the modalities C12 and C6 registered in the two last weeks a decrease in their concentrations. An increase in C2 modality was observed along all the ripening (Fig. 2b).

For Tinta Roriz seeds, same fluctuations occurred in R12 and R6 modalities, however a tendency to their decrease was presented specially by R12 modality. As for R6 modality a decrease was observed all along ripening. About skins we observed that, all modalities showed a peak 18 days after veraison, suffering afterwards a pronounced decrease until the last two weeks, followed by a stabilisation with slight fluctuations in all modalities. For the pulp R6 and R2 modalities presented a pronounced decrease in the first two weeks after veraison, time when the minimum values were found. Afterwards these modalities showed an increase until the end of ripening. R12 modality was characterized by showing pretty much the same values along all the ripening (Fig. 3b).

Polymeric fraction

In what concerns the polymeric fraction in seeds and for all modalities of Cabernet Sauvignon, a very identical profile along all ripening was noticed. The skin presented in C6 and C2 modalities a peak of concentration 25 days after veraison, having decreased afterwards until the end of ripening. Modality C12 suffered a decrease until the last two weeks of ripening and from this time onwards until the end, an increase in its concentration was observed. For the pulp of Cabernet Sauvignon we observed, that all modalities showed a decrease along the ripening except for modality C2 that presented in the last two weeks a pronounced increase of this fraction (Fig. 2c). According to Sun *et al*. (2001), proanthocyanidins presented in the pulp may have resulted by contaminations when the separation of the pulp from the skin was done. Therefore, the increase in monomeric, oligomeric and polymeric fractions in the pulp of the 75% pruning modality of Cabernet Sauvignon can have been due to this contamination since the berries had achieved a higher degree of ripeness making the separation of pulp and skin more difficult, thus increasing the contamination degree.

For Tinta Roriz we noticed that in seeds, R12 and R6 modalities presented

a similar evolution until the 32º day after veraison and from then onwards R2 modality suffered a very pronounced decrease and R12 modality remained practically stable. R6 modality presented a peak 18 days after veraison decreasing afterwards until the end of ripening.

For the skins, R12 and R6 modalities presented a nearly identical profile, showing a minimum value of concentration on the 32º day after veraison, followed by an increase until the end of ripening. Modality R6 also presented a minimum value on the 32º day, but the following increase was much more pronounced in this modality. In Tinta Roriz the pulp presented for R12 modality an increase along all ripening. R6 modality remained relatively constant along the ripening process and R2 modality was characterized by showing fluctuations along ripening suffering a decrease in the last two weeks, just the opposite of what happened with the other modalities (Fig. 3c).

Global overview: The tendency for higher concentrations of condensed tannins in the early stages of berry development may be relational with their metabolization throughout the ripening. The pattern of decline after veraison of seeds as suggested by Kennedy *et al*. (2000 b) could be explained by oxidation reactions. According to Cheynier *et al.* (1997) this decrease could be attributed to reduced extractability resulting from the conjugation of proanthocyanidins with other cellular components. Such a decrease in berry proanthocyanidins post veraison has been previously reported by various authors (Czochanska *et al*.,1979; Kennedy *et al*.,2000 a,b; Harbertson *et al*.,2002; Downey *et al*.,2003). In addition, we noticed that for all tannin fractions, and for all modalities, skins of Tinta Roriz presented higher tannins concentrations than Cabernet Sauvignon.

Fig. 4 shows that at harvest for all the berry compounds (in skins, seeds and pulp), on both varieties and respective modalities, the monomeric fraction (F1) was the one that presented minor concentration, and the polymeric (F3) the one that presented major concentration of condensed tannins. These results confirm the investigations of Sun *et al*. (1998 a; 2001) in pulp, skins and seeds and Jordão *et al.* (2001), also in grape bunch.

Tab. II and III shows that at harvest, for both varieties and respective pruning modalities seeds presented the major concentration of all tannins followed by skins and pulp, confirming other works (Bourzeix *et al.* 1986; Ricardo-da-Silva *et al.* 1992 a; Hmamouchi *et al*. 1994; De Freitas *et al*. 2000; Jordão *et al*. 2001 a,b ; Sun *et al.* 2001 ; Downey *et al*. 2003).

An interesting result for the pulp polymeric fraction (F3) was observed at harvest. In the case of Cabernet Sauvignon an increased in this fraction was observed with the level of fruit pruning and the opposite occurred with Tinta Roriz variety (Fig. 4).

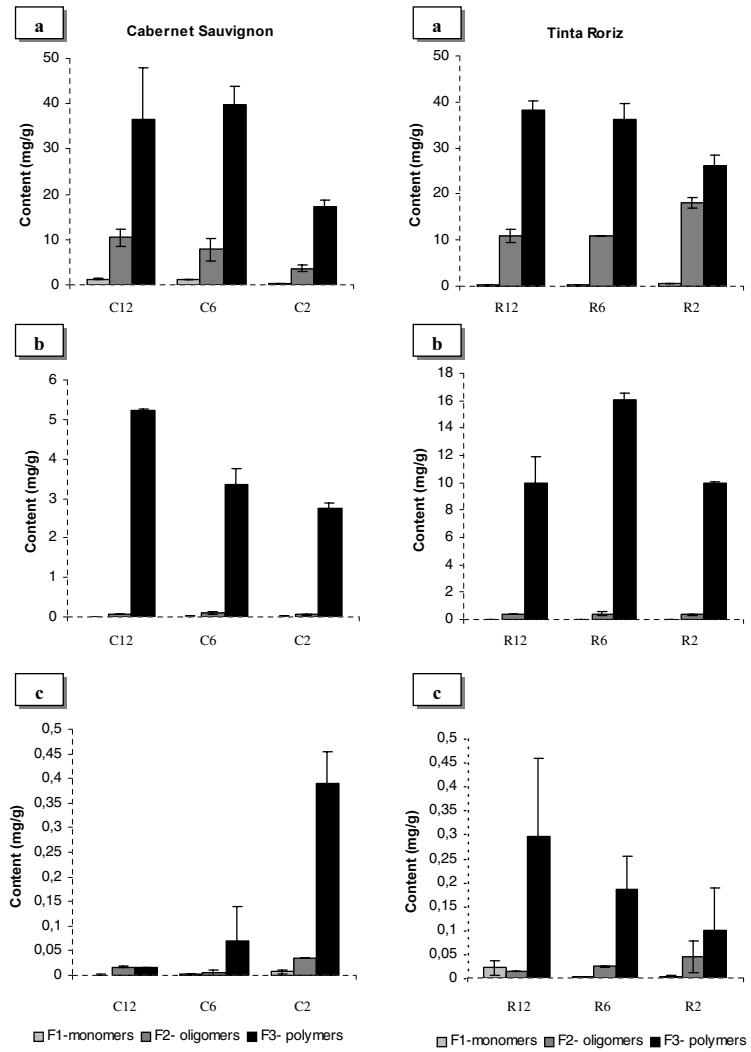

Figure 4. - Flavan-3-ol content of monomeric (F1), oligomeric (F2) and polymeric (F3) fractions at harvest of (**a**) seeds, (**b**) skins and (**c**) pulp, from Cabernet Sauvignon (1 Oct. 2002) (**C12**: witness modality, **C6**: 50% pruning modality, **C2**: 75% pruning modality) and Tinta Roriz (23 Sept. 2002) (**R12**: witness modality, **R6**: 50% pruning modality, **R2**: 75% pruning modality). Bars represent the standard deviation.

*Concentracção taninica (mg/g) registada à vindima para a fracção monomérica (F1), oligomérica (F2) e polimérica (F3) da (**a**) grainha, (**b**) película e (**c**) polpa na casta Cabernet Sauvignon (1 Out. 2002) (**C12**: modalidade testemunha, **C6**: modalidade com 50 % de monda, **C2**: modalidade com 75 % de monda) e da casta Tinta Roriz (**R12**: modalidade testemunha, **R6**: modalidade com 50 % de monda, **R2**: modalidade com 75 % de monda). As barras representam o desvio padrão.*

Table II

Monomeric (F1), Oligomeric (F2) and Polymeric (F3) tannin concentration (mg/g) of seeds, skins and pulp of different pruning modalities of red cv. Cabernet Sauvignon (**C12**: witness modality, **C6**: 50% pruning modality, **C2**: 75% pruning modality) at harvest.

*Concentracção taninica (mg/g) registada à vindima para a fracção Monomérica (F1), Oligomérica (F2) e Polimérica (F3) da grainha, película e polpa na casta Cabernet Sauvignon (**C12**: modalidade testemunha, **C6**: modalidade com 50 % de monda, **C2**: modalidade com 75 % de monda).*

1 Oct. 2002		C12	C6	C2
Monomeric	**Seeds**	1,228a	1,148a	0,228b
	Skins	0,003a	0,007a	0,013a
	Pulp	0,001a	0,003ab	0,007b
Oligomeric	**Seeds**	10,426a	7,840a	3,586ab
	Skins	0,066a	0,099a	0,057a
	Pulp	0,017a	0,005a	0,034a
Polymeric	**Seeds**	34,427a	39,716a	17,369b
	Skins	5,238a	3,351b	2,750b
	Pulp	0,015a	0,069a	0,351b

Separation of mean values by LSD test with a significance level of 5%.
Note: Means followed by the same letter are not significantly different ($p<0,05$).

Separação das médias pelo teste LSD com um nível de significância de 5%.
Nota: Os valores com a mesma letra não diferem significativamente entre si.

Table III

Monomeric (F1), Oligomeric (F2) and Polymeric (F3) tannin concentration (m/g) of seeds, skins and pulp of different pruning modalities of red cv. Tinta Roriz (**R12:** witness modality, **R6**: 50% pruning modality, **R2**: 75% pruning modality) at harvest.

*Concentracção taninica (mg/g) registada à vindima para a fracção Monomérica (F1), Oligomérica (F2) e Polimérica (F3) da grainha, película e polpa na casta Tinta Roriz (**R12**: modalidade testemunha, **R6**: modalidade com 50 % de monda, **R2**: modalidade com 75 % de monda)*

23 Sept. 2002		R12	R6	R2
Monomeric	**Seeds**	0,270a	0,159a	0,564b
	Skins	0,002a	0,018a	0,019a
	Pulp	0,004a	0,002a	0,004a
Oligomeric	**Seeds**	10,871a	10,957a	18,115b
	Skins	0,378a	0,419a	0,343a
	Pulp	0,013a	0,024a	0,044a
Polymeric	**Seeds**	38,204a	36,266a	13,111b
	Skins	9,939a	16,057b	9,954a
	Pulp	0,295a	0,186a	0,099a

Separation of mean values by LSD test with a significance level of 5%.
Note: Means followed by the same letter are not significantly different ($p<0,05$).

Separação das médias pelo teste LSD com um nível de significância de 5%.
Nota: Os valores com a mesma letra não diferem significativamente entre si.

At harvest, in what concerns these compounds, the most relevant results show a significant difference between the control and the other two pruning modalities, namely a lower concentration of polymeric condensed tannins fractions in seeds, skins and pulp, for both vine varieties except for the pulp in Cabernet Sauvignon (Fig. 4).

CONCLUSIONS

Along the ripening, a tendency to the decrease of all condensed tannins whatever the degree of polymerization in all berry components (pulp, skins and seeds) in the two vine varieties studied (Cabernet-Sauvignon and Tinta Roriz) and respective modalities of fruit pruning (C12, C6, C2, R12, R6, R2).

At harvest, several major analytical parameters of grape berries of the two varieties studied and for all modalities of fruit pruning performed were not significantly changed.

The experiment must be repeated in different climatic and soil conditions and with averages of several years, but this results in a certain way, allow us to question on the eventual interest of fruit pruning, at least to the level of its effect in the principal analytic parameters of the berries, except on the condensed tannins.

RESUMO

Alterações ao longo da maturação nos taninos condensados da grainha, película e polpa: influência da monda de cachos

O presente trabalho teve por objectivo estudar a evolução ao longo da maturação dos taninos condensados segundo o seu grau de polimerização na grainha, polpa e película, nas uvas das castas Cabernet Sauvignon e Tinta Roriz (*Vitis vinifera* L.).

Para este estudo comparou-se uma testemunha não mondada com uma modalidade onde foi realizada uma monda de 50 % de cachos e uma outra modalidade com 75 % de monda.

A quantificação dos taninos condensados segundo o seu grau de polimerização foi realizada por fraccionamento em cartuchos C_{18} Sep-Pak, seguida da reacção com a vanilina e também por HPLC.

Observou-se uma tendência ao longo da maturação para o decréscimo dos taninos condensados qualquer que seja o seu grau de polimerização para todos os constituintes do bago (polpa, grainha e película), para as duas castas e respectivas variedades.

Não foram registadas na amostragem de vindima diferenças significativas nos parâmetros analíticos estudados nas castas Cabernet Sauvignon e Tinta Roriz e respectivas modalidades. No que concerne aos taninos condensados o resultado mais relevante foi a diferença significativa entre a testemunha e as outras duas modalidades de monda, nomeadamente ao nível da fracção polimérica que apresentou na grainha, película e polpa da testemunha, uma concentração mais baixa.

RÉSUMÉ

Evolution des tannins condensés des pépins, des pellicules et de la pulpe pendant la maturation du raisin: effet de l'éclaircissage des raisins

Le but de ce travail est l'évolution tout au cours de la maturation des tanins condensés d'après son degré de polymérisation dans la pellicule, le pépin et la pulpe des raisins des cépages Cabernet Sauvignon et Tinta Roriz (Vitis vinifera L.)

On a confronté un témoin de chaque cépage non taille avec une modalité avec une charge de fruits à la taille de 50 % et avec une autre de 75 % du charge.

Le fractionnement des tanins condensés avec différent degré de polymérisation a été réalisé en cartouches C_{18} Sep-Pak suivi de la réaction avec la vanilline, et aussi par HPLC.

On a enregistré tout au cours de la maturation une tendence vers le décroissement des tanins condensés indépendamment du degré de polymérisation de tout des constituants du grain (pellicule, pulpe et pépin), avec les deux cépages et respectives modalités. Au vendange, suivant les différentes modalités de taille de raisins et pour les deux cépages étudiés (Cabernet Sauvignon et Tinta Roriz), on n'a pas enregistré de modifications dans les principaux paramètres analytiques. En ce que concerne les tanins condensés, le résultat le plus significatif est la différence importante entre le témoin et les deux modalités de taille, notamment au niveau de la fraction polymère dans les pépins, la pulpe et les pellicules que a obtenue, dans ce témoin, les valeurs les plus basses.

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