PHENOLIC EXTRACTION DURING FERMENTATION AS AFFECTED BY RIPENESS LEVEL OF SYRAH/R99 GRAPES
INFLUENCE DE LA MATURITÉ DU RAISIN SUR L'EXTRACTION PHÉNOLIQUE AU COURS DE LA FERMENTATION ALCOOLIQUE DU CÉPAGE SYRAH/R99

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Key words: Grapevine, Shiraz, fermentation, phenols, ripeness level, skins, seeds, wine

Abstract
Phenolic (tannin and anthocyanin) extraction during fermentation of Syrah grapes was investigated as part of an elaborate study to determine parameters that would indicate high grape quality and different grape and wine styles. A Syrah/R99 vineyard, situated in the Stellenbosch region (South Africa), was used. Vines are vertically trained and spaced 2.75 x 1.5 m in north-south orientated rows on a Glenrosa soil and a west-facing slope. Microsprinkler-irrigation was applied at pea berry size and at vèraison stages. Canopies were suckered, shoot-positioned and topped, whereas leaves were removed at two stages. Fortnightly sampling was done from berry set up to two weeks post-véraison, after which grapes were harvested for analyses and winemaking approximately every four days. Six wines were made per ripeness level.

Results obtained during the ripening period of the 2002/2003 growth season (from 17 February to 24 March) are reported. Whole berries, skins, seeds, pomace and wine were analysed for each ripeness level. Grapes of all harvests were cooled to the same temperature (20 ºC) before processing. Grapes were destemmed, crushed and the pomace inoculated with commercial yeast (VIN 13). Alcoholic fermentation took place at a controlled temperature of 24 ºC (di-ammonium phosphate and SO_2 were added). The skins were pushed through three times per day. Fermentation on the skins averaged five days, after which the pomace was pressed. Skins and juice were analysed on the first, second and fourth day during fermentation. On the fifth day after crushing (at pressing), skins, juice and seeds were analysed. Total soluble solids, titratable acidity, pH, anthocyanins, tannins and phenolics were analysed in the whole berries. Evolution of colour density ($A_{520 + 420}$) and total phenolic content (absorbance at 280nm) was monitored in the pomace and skins. Proanthocyanidin content (DMAC analysis) was determined in the seeds from intact berries and in the seeds after pressing. The degree of alcohol, phenolics, colour intensity and colour density were determined in the different wines.

The ^{0}Balling of the berries reached a high at approximately 11 March (178 days after bud burst). This pattern was similar to that of the anthocyanin, tannin and total phenolic contents of the berry (whole berry extraction) and coincided with the reduction in berry size due to water loss. After 11 March extraction of the different phenolic compounds seemed not to be affected by the decrease in berry size. From 17 March no further extraction from the skins (skin extraction after 5 days of fermentation) occurred, hence the stable colour density and total phenolic patterns of the skins during this period. The colour density and total phenolic content of the skins during fermentation showed a clear distinction between harvest dates with higher extraction occurring from 11 March to the last harvest date, resulting in low remaining values in the skins after five days of fermentation. The proanthocyanidin content of the seeds only slightly decreased during the course of ripening. However, the seeds were heavily depleted during fermentation of the harvests following that at approximately 6 weeks after véraison, a trend which is completely opposite to the sugar content of the berries. The colour density and total phenolic content of the wine followed similar patterns to those of the berries.

Resumé
L'extraction phénolique au cours de la fermentation à partir de vendanges de différents degrees de maturité du cépage Syrah/R99 a été etudiée. Cette travail fait parti d'un projet focalisé sur la qualité du raisin et des vins obtenus au cours du millésime 2002. Les vignes sont situées à Stellenbosch (Afrique du Sud) sur un sol Glenrose avec un système de conduite en palissage vertical; la distance entre les rangs orientés nord-sud est de 2.75 m et de 1.5 m entre les souches. Les plantes ont été irriguées par microaspersion après la fermeture de la grappe (baie petit pois) jusqu'à la véraison. Six échantillons de baies ont été prélevés depuis la nouaison jusqu'à deux semaines après la véraison, date qui signale le début des vendanges pour les différents degrés de maturité. Les souches ont été vendangées en six bloques chaque quatre jours puis les raisins sont immédiatement vinifiés.

Les résultats correspondent aux périodes s'échelonnant entre le 17 février et le 24 mars 2002. Les analyses de la baie entière, de l'épiderme et des pépins ont été réalisées sur les raisins et les vins des 6 bloques expérimentaux. Les raisins sont placés à la même temperture de 20ºC avant le début de la fermentation. Les vendanges après être éraflées, foulées et sulfitées sont additionnées d'activateurs puis inoculées par *S. cerevisiæ* (VIN 13) et fermentent à la tempertaure constante de 24ºC. La durée moyenne de la vinification (fermentation alcoolique + macération postfermentative) a été de 5 jours. Trois pigeages ont été effectués par jour jusqu'au cinquième jour temps où le pressurage est réalisé. Des échantillons d'épiderme de raisins et du moût en fermentation ont été analysés le premier, deuxième, quatrième et cinquième jour après le pressurage. La concentration en sucre, l'acidité totale, le pH, les teneurs en anthocyanes et tanins ainsi que les polyphénols totaux (IPT) sont analysés. De plus, pendant la fermentation, l'évolution de densité colorante ($A_{520 + 420}$) et de l'IPT (A_{280}) est aussi déterminée. Les proanthocyanidins selon l'analyse du DMAC ont été déterminés dans les pépins des baies et pendant la vinification. Dans les vins, sont analysés le degré alcoolique, l'intensité et la densité colorante, et les polyphénols totaux.

Le contenu maximum de sucre se situe autour du 11 mars (178 jours après le débourrement). Ce maximum d'accumulation est aussi observé pour les anthocyanes, les tanins et les polyphénols totaux des baies, accompagné en parallèle d'une diminution du volume de la baie due à la perte d'eau. Après l'onze de mars, l'extraction des polyphénols totaux n'est pas en relation avec la taille de la baie. L'extraction des polyphénols totaux de l'épiderme au cinquième jour de fermentation est totale après le 17 mars et par la suite la densité colorante et les phenols (IPT) dans l'épiderme se maintiennent constants après cette période. Si on compare les différents degrés de maturité, l'extraction augmente de forme notable après l'onze de mars et l'on observe une diminution plus marquée de la densité colorante et de l'IPT des épidermes le cinquième jour de la fermentation. Le contenu des catéquines dans les pépins n'a pas beaucoup changé pendant la maturation des baies alors que la diminution à la fin de la fermentation pour les pépins de raisins récoltés 6 semaines après la véraison a été très importante, contraire à l'accumulation des sucres. Les valeurs de densité colorante et IPT des vins suivent un modèle similaire à celles trouvées dans les baies.

Introduction

The colour, structure, and sensorial properties of red wine are mainly influenced by quality substances known as phenolic compounds. For this reason, both their synthesis and concentration during growth and maturity of the berry as well as its evolution during the winemaking and ageing processes have been studied by many research groups.

Climate, soil, cultivation and biology are some of the most important factors that affect synthesis and concentration of phenols in berries. Accumulation of phenolic compounds is dependent on soil type and fertility (Carbonneau, 2000; Barbeau *et al*., 2001), inherent soil water holding capacity, and the annual precipitation of the specific terroir (Esteban *et al*., 2001; Choné *et al*., 2001). These factors significantly affect the vigour and production of the plant. Cultivation techniques, such as trellising system, pruning method and canopy management are critical for optimal functioning of the canopy and grapes and establishment of a balance between vegetative and reproductive growth (Smart, 1990; Hunter *et al*., 1995). Furthermore, both photosynthetic active radiation (PAR) and phenyl alanine ammonia lyase (PAL) activity are essential for the synthesis of phenols in berries (Roubelakis-Angelakis *et al*., 1986). Together with the ratio of skins and seeds to berry size, extraction of

phenolics and the final level and nature of the phenolic concentration of the wine will be determined (Roson & Moutonet, 1992).

The total berry phenolic concentration slowly increased during maturation until a maximum is reached one or two weeks before harvest, depending on variety and climatic conditions (Gonzalez San José *et al*., 1990; Jordao *et al*., 1998; Vivas *et al*., 2001; Habertson & Adams, 2002). Before veraison, there was no significant increase of phenolic compounds in the berries. The concentration of tannins, phenols synthesised during development of the green berries, decreased during the ripening period (Kennedy *et al.*, 2001, Ojeda *et al*., 2002; Valls, 2004). The catechins and esters of hydroxycinnamic acids in the skin decreased because of the berry size increase. In the seeds, this decrease could also be attributed to oxidation processes (Kennedy *et al*., 2000). During veraison the rate of anthocyanin synthesis increased significantly (Lamaridis *et al*., 1997), contributing to the total increase in phenolics during this time.

Grape characteristics, molecular structure of the anthocyanins, must composition and extraction conditions affect the concentration and stability of the colour, the astringency and tannin structure of the wine as well as its ageing potential (Zoecklein, 1991; Di Stefano *et al*., 1994; Mazza, 1995; Vivas *et al*., 2001). During pomace maceration in the red winemaking process, phenolic compounds are extracted due to a diffusion process. The extraction profile is characterised by an exponential phase during the first stage, followed by a stationary phase where no more phenols are extracted, even when this process is extended. The vinification technique may cause a change in the nature of the anthocyanins. The acylated derivates generally decrease during the winemaking process and this decrease is more significant with intensive extraction techniques (La Notte *et al*., 1992). Extraction of the flavan-3-ols and its proanthocyanidin oligomers progressively increase during alcoholic fermentation and pomace maceration. Its diffusion into the must mainly depends on the variety and the conditions of extraction (Scudamore *et al*., 1990; Karuamanchir *et al*., 1996; Souquet *et al*., 1996).

In this study, we report on the extraction of phenolic (tannin and anthocyanin) compounds from the skins and seed during fermentation of Syrah grapes harvested at different ripeness levels. This formed part of an elaborate study to determine parameters that would indicate high grape quality and different grape and wine styles.

Materials and Methods

Vineyard: A Syrah/R99 vineyard, situated in the Stellenbosch region (South Africa), was used during the 2002/03 growth season. Vines are vertically trained and spaced 2.75 x 1.5 m in north-south orientated rows on a Glenrosa soil and a west-facing slope. Microsprinkler-irrigation was applied at pea berry size and at vèraison stages. Canopies were suckered, shoot-positioned and topped, whereas leaves were removed at two stages (Hunter, 2000). Fortnightly sampling was done from berry set up to two weeks post-véraison, after which harvesting for grape analyses, wine making and wine analyses was done approximately every four days (from 17 February to 24 March). Six wines were made per ripeness level.

Analyses and winemaking: Whole berries, skins, seeds, pomace and wine were analysed for each ripeness level. Grapes of all harvests were cooled to the same temperature (20 ºC) before processing. Grapes were destemmed, crushed and the pomace inoculated with commercial yeast (VIN 13). Alcoholic fermentation took place at a controlled temperature of 24 ºC (di-ammonium phosphate and SO_2 were added). The skins were pushed through three times per day. Fermentation on the skins averaged five days, after which the pomace was pressed. Skins and juice were analysed for anthocyanins, tannins, total phenolics (A_{280}) and colour density ($A_{520 + 420}$) (Ribéreau-Gayon *et al*., 2000) on the first, second and fourth day during fermentation. On the fifth day after crushing (at pressing), seeds were analysed for proanthocyanidins by the DMAC method according to Vivas *et al*. (1994). The same analysis was performed on the skins and juice (wine). Proanthocyanidin content (DMAC analysis that determine catechins and the oligomers) was determined in the seeds from intact berries and in the seeds after pressing. Total must soluble solids, titratable acidity, and pH were analysed according to standard methods, whereas anthocyanins, tannins and phenolics were analysed in the whole berries and wines according to Ribéreau-Gayon *et al*. (2000). The degree of alcohol, total phenolics (A_{280}) and colour density ($A_{520 + 420}$) were also determined in the different wines.

Results and discussion

The ^{0}Balling of the berries reached a high at approximately 11 March (178 days after bud burst). This pattern was similar to those of the anthocyanin, tannin and total phenolic contents of the berry (whole berry extraction) and coincided with the reduction in berry size due to water loss (Figs 1 & 2). During the last three harvests (after 11 March), extraction of the different phenolic compounds seemed not to be affected by the decrease in berry size (higher skin:pulp ratio). Anthocyanin and tannin contents of the whole intact berry stabilised, whereas the total phenolic content of the berry only slightly increased at the last harvesting date. Total phenolic extraction from the skins (skins analysed at the end of fermentation) reached a maximum at 11 March, after which no further extraction occurred. This is also evident from the stable skin colour density that occurred from 17 March.

The colour density and total phenolic content of the skins during fermentation showed a clear distinction between harvest dates with higher extraction occurring from 11 March to the last harvest date (Figs 3 & 4). The results from eight harvests (17 February to 24 March) showed low values after five days of fermentation, particularly for the three latest harvests. Two different patterns were observed; skins lost less colour at the beginning of the maturation period (dates between 17th of February and 7th of March) than towards the end. Total phenolic values (absorbance 280) in the skins were between 25 and 30 in the less ripe harvests and then decreased to values of less than 25 during the last three harvests (from 11 to 24 March). This is also illustrated by the similar skin colour density pattern.

Except for the harvest of 17 February, the colour density and total phenolic content of the wine followed similar, but opposite, patterns than those found in the skins, the three last harvests showing higher values than the rest (Fig. 5). The maximum level of total wine phenolics was reached at the last harvest (24 March). In all cases, lower phenolics and colour density values were obtained on the fifth day at pressing. The decrease in phenolic content at the end of fermentation could also be due to different combinations (Singleton & Trousdale, 1992; Di Stefano *et al*., 1994) or polymerisations which stabilise the wine (Mayen *et al*., 1994). The colour density and total phenol content of the wine followed similar patterns to those of the berries (Fig. 6).

The proanthocyanidin content of the seeds only slightly decreased during the course of ripening up to 11 March (Fig. 7). However, the seeds were heavily depleted during fermentation of the harvests following that at approximately 6 weeks after véraison, a trend which is completely opposite to the sugar content of the berries. The decrease of proanthocyanidins in seeds could be explained by an increase in the degree of polymerisation from 3 March to 17 March. The slight increase in alcohol in the wine from the latest harvests is not an explanation for the heavy depletion of proanthocyanidin content in seeds. It seemed that the extraction from seeds is not related to the alcohol content in wines during fermentation (Fig. 7, left). Kennedy *et al*. (2000) found a decrease in the degree of polymerisation in seeds (in berries) during the ripening process, while Downey *et al*. (2003) observed an increase. Our results did not show big differences along ripeness.

In the wine, proanthocyanidins analysed during fermentation of six harvests (from 25 February to 24 March) showed two different patterns of extraction (similar to what was found for skins). Contents were higher at the three latest harvests, reaching the maximum at the fourth day of fermentation (Fig. 8). A decrease at the fifth day of fermentation was observed in all cases, being higher for 11 and 17 March harvests and lower for the 24 March harvest.

Conclusions

In over-ripe grapes, phenol extraction from the skins is limited, despite an increase of values in the wine. However, more extraction of flavan-3-ols from the seeds may occur in such grapes, explaining the values obtained in wine. Polymerisation of phenolics in seeds increased according to ripeness level, but was restricted in over-ripe grapes. It can be concluded that the ripeness level of the skins affect the phenol content of the wine and the ripeness level of the seeds affect the nature of phenols in the wine. The results obtained on phenolic patterns during fermentation were perfectly matched with wine quality.

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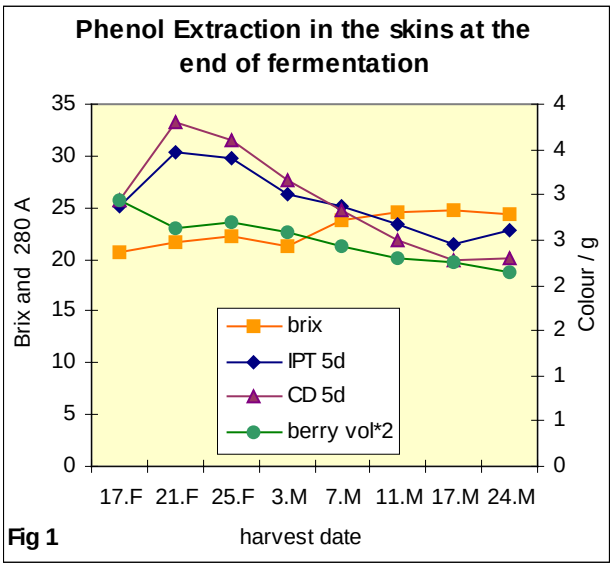

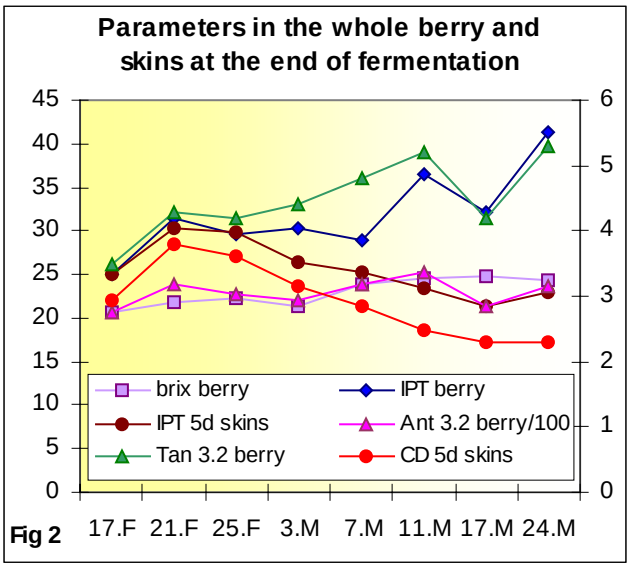

Figure 1. Phenol extraction related to Brix and berry mass/ **Figure 2.** Phenol parameters in the whole berry and in the skins analysed at the end of fermentation. CD= colour density; IPT = A_{280}; Ant= anthocyanins; Tan= tannins; 5d= 5th day, the end of alcoholic fermentation (after pressing). F=February; M= March.

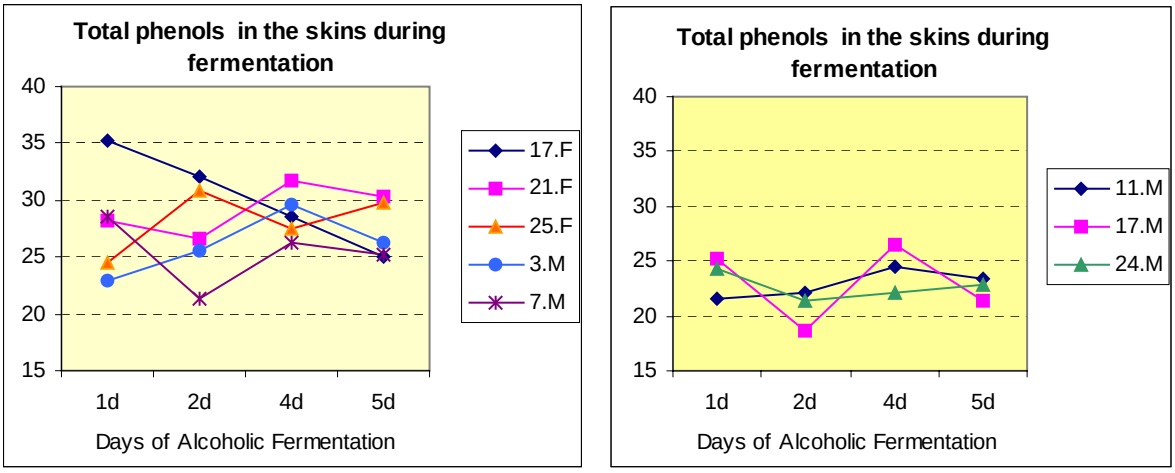

Figure 3. Evolution of the IPT (Index of Total Phenols, A_{280}) during alcoholic fermentation at 8 different ripeness levels. The sample of the 5th day was taken just after pressing.

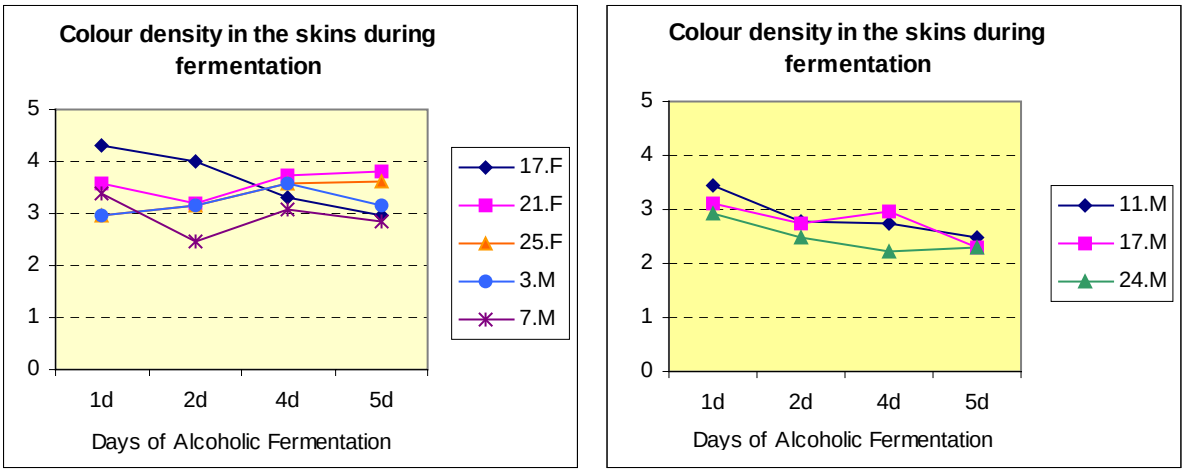

Figure 4. Evolution of colour density ($A_{420+520}$) during alcoholic fermentation at 8 different ripeness levels. The sample of the 5th day was taken just after pressing.

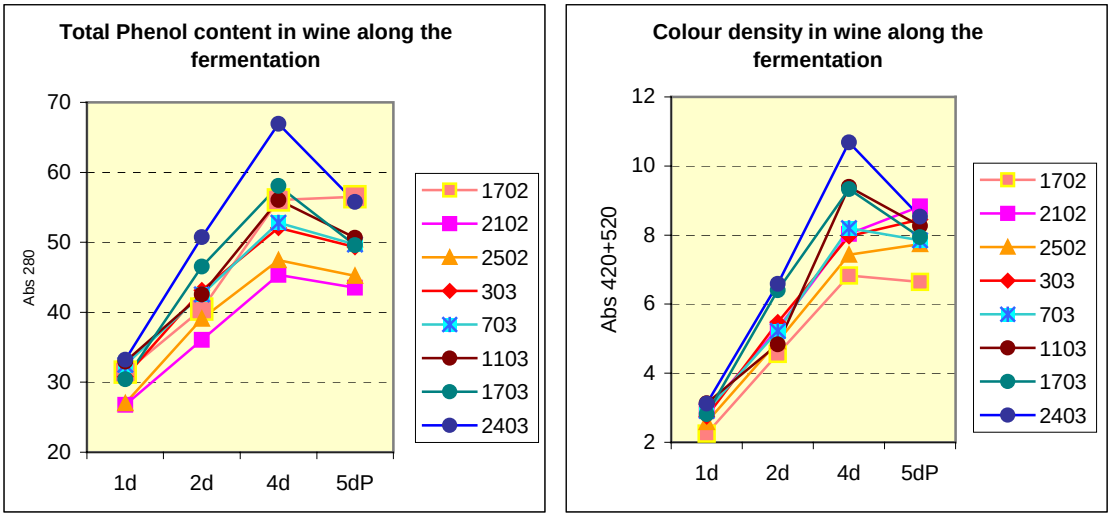

Figure 5. Evolution of total phenol content in wine (absorbance at 280) and colour density ($A_{420+520}$) during alcoholic fermentation at 8 different ripeness levels. The sample 5dP corresponds to the 5th day just after pressing.

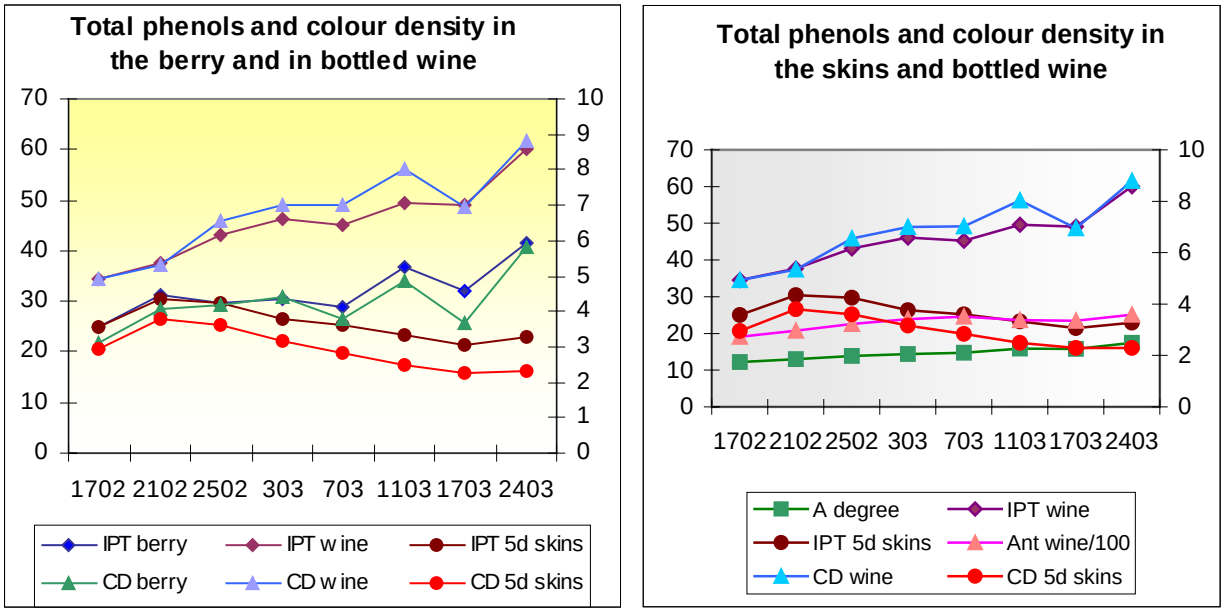

Figure 6. Evolution of total phenol content (A_{280}) and Colour density ($A_{420+520}$) in wine related to the berry (left graph) and skins (right graph).

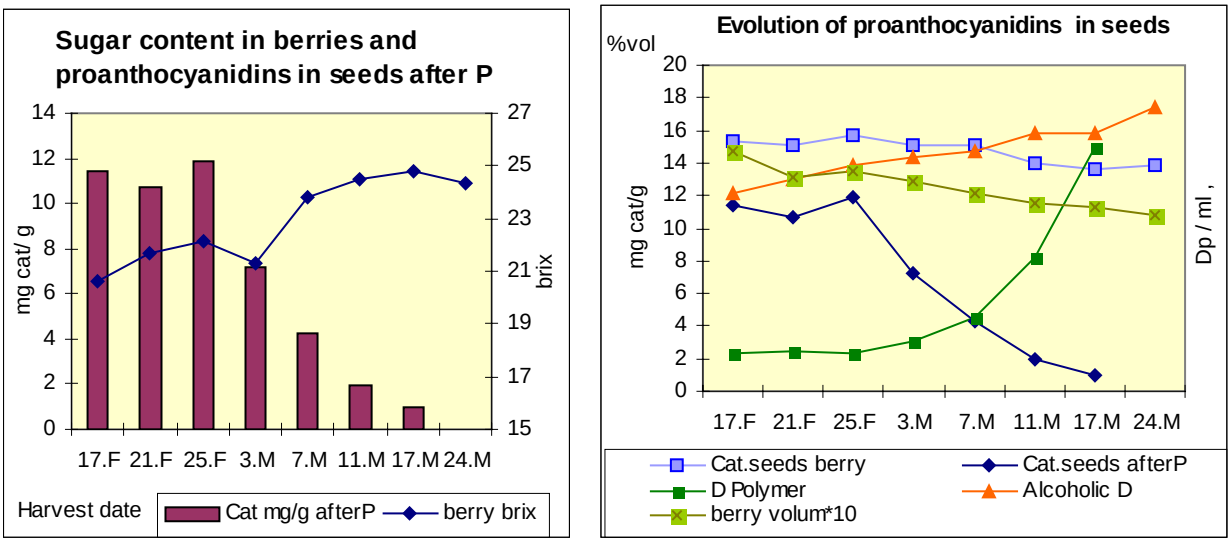

Figure 7. Left graph: Proanthocyanidin content in seeds after pressing and the level of soluble solids in berries. The right graph represents the proanthocyanidin content in the whole berries at different ripeness level and in the seeds at the fifth day after pressing, the degree of polymerisation, berry volume and alcoholic degree of the wine (%vol). F=February; M= March.

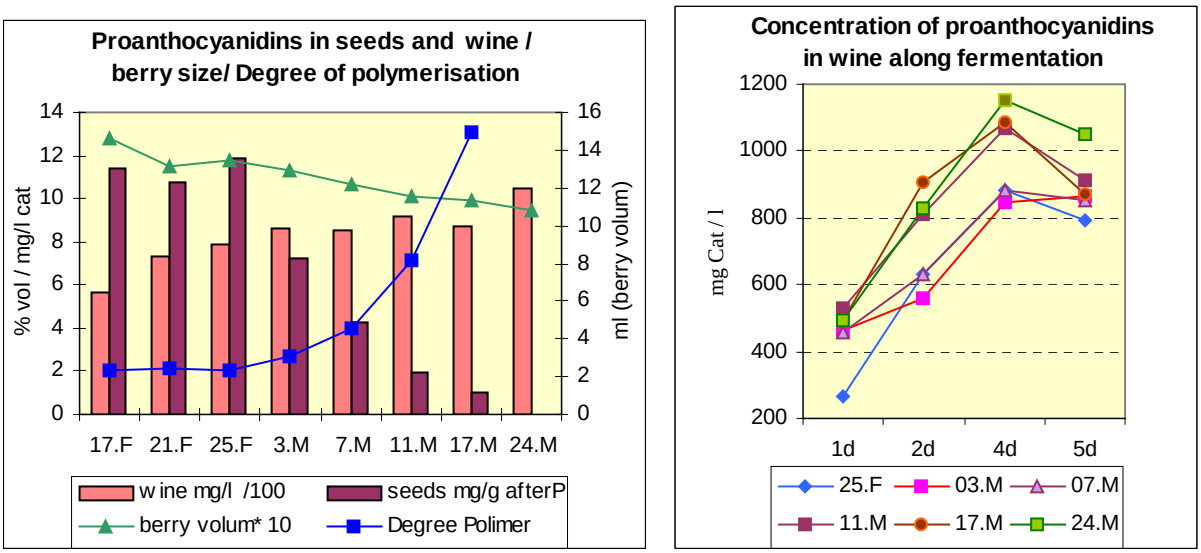

Figure 8. Left graph: Proanthocyanidins in seeds and wine related to the degree of polymerisation and to the berry size. The right graph represents the proanthocyanidins during fermentation. F=February; M= March.