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# Effect of red wine maceration techniques on oligomeric and polymeric proanthocyanidins in wine, cv. Blaufränkisch

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## Summary

**The influence of duration of maceration, of different maceration temperatures during the last two days of maceration, and the effect of malolactic fermentation on the content of oligomeric and polymeric proanthocyanidins in wine, cv. Blaufränkisch, were investigated using spectrophotometric methods and the NP-HPLC procedure. Results show that the duration of maceration affected the extraction from seeds more strongly, while heating of must primarily affected the extraction from skins. An increase of temperature from 25 °C to 35 °C during the last two days of maceration increased total anthocyanins and high molecular weight proanthocyanidins. Malolactic fermentation lowered the color of wine, the amount of total anthocyanins and total polyphenols and may reduce polymerization reactions of proanthocyanidins.**

K e y w o r d s : wine, polyphenols, proanthocyanidins, maceration, malolactic fermentation.

## Introduction

The polymerization of flavan 3-ols produces a class of oligomeric and polymeric polyphenols called proanthocyanidins (condensed tannins). Proanthocyanidins are important components of wine because they are responsible for significant organoleptic properties, primarily bitterness, astringency, and the body of wine. It has also been shown that they have potent antioxidant and free radical scavenging abilities (Ricardo da Silva *et al.* 1991; Rigo *et al.* 2000). Organoleptic and pharmacological properties of proanthocyanidins largely depend on the structure and especially on the degree of polymerization (Rigaud *et al.* 1993). The low molecular weight molecules are mainly bitter, whereas the high molecular weight proanthocyanidins are astringent (Lea and Arnold 1978; Arnold *et al.* 1980). Polymerization between anthocyanins and proanthocyanidins is important for stabilizing wine color by protecting the anthocyanins from bleaching. Polymerization of anthocyanins with tannins is also important for the maintenance of tannins in wine (Singleton and Trousdale 1992). It has been suggested that the beneficial effects for health of these compounds are depending on the degree of polymerization (Saito *et al.* 1998).

Red wine color and taste are largely related to the phenolic compounds extracted from the grape during winemaking. Winemaking protocols significantly affect the phenolic composition of wine. The extraction rate depends on various factors, including concentration of the proanthocyanidins in grape, maceration time, maceration temperature, cap punching program, levels of alcohol, and $SO_2$ concentration. As maceration time increases, seeds play an increasingly important role as a source of proanthocyanidins (Singleton and Draper 1964). Some authors have observed that seeds can contribute significant amounts of the proanthocyanidins to red wine (Kovac *et al.* 1995; Nicolini *et al.* 1998), while others reported the opposite (Cheynier *et al.* 1989; Ricardo da Silva *et al.* 1993). Catechins and oligomeric proanthocyanidins have recently been shown to be extracted mainly from grape seeds while grape skins and stems are important sources of polymeric proanthocyanidins for wine (Sun *et al.* 1999). Thorngate and Singleton (1994) reported that almost all monomeric flavan-3-ols are found in the outer seed coat and endosperm, while the proanthocyanidins are mostly localized in the brown hull. Therefore, their extraction is slower.

The most commonly used analytical methods are either spectrophotometric assays for determination of different classes of oligomeric and polymeric proanthocyanidins (Di Stefano *et al.* 1989; Rigo *et al.* 2000) or RP-HPLC methods for determination of low molecular weight polyphenols (Lamuela-Raventós and Waterhouse 1994). Recently some effective HPLC methods for the separation of the proanthocyanidin oligomers and polymers by normal phase techniques have been described (Rigaud *et al.* 1993; Hammerstone *et al.* 1999; Lazarus *et al.* 1999; Kennedy and Waterhouse 2000).

The aim of the present work was to compare the influence of maceration time and maceration temperature during red winemaking on the content of oligomeric and polymeric proanthocyanidins. The effect of malolactic fermentation was also investigated. These trials were performed with the cv. Blaufränkisch, an important variety in Slovenia and central Europe (Ambrosi *et al.* 1994).

## Material and Methods

Y e a s t s t r a i n s a n d m a l o l a c t i c b a c t e r i a : *Saccharomyces cerevisiae*, Fermicru VR 5, Gist-brocades, Seclin Cedex, France, and BioStart oenos (*Oenococcus oeni* SK1), Erbslöh, Geisenheim, Germany, were used.

G r a p e s : In 1998 and 1999 grapes of the cv. Blaufränkisch were harvested at technological maturity, in the Posavje region (District of Bela Krajina, Slovenia).

W i n e s . 1 9 9 8 t r i a l s : A total of 500 kg of grapes was destemmed, crushed, and divided into 4 homogeneous

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batches (125 kg of must each). 20 mg·l$^{-1}$ of $SO_2$ (5 % $H_2SO_3$) were added to the mash prior to fermentation. All batches were inoculated with yeast (20 g dry wt ·hl$^{-1}$), and punched down three times a day during active fermentation for 5 d. The duration of maceration or contact between solids and liquid, was 12, 15, 19 and 24 d at 25 ºC. At the end of maceration the free run was drained and the pomace was pressed at 1.5 bar in a tank membrane press. The free run and press run of each lot were combined. The wines were stored in 100 l stainless steel tanks at room temperature for 5 months, racked three times during storage. 50 mg·l$^{-1}$ of $SO_2$ (5 % $H_2SO_3$) were added to the wines at the end of malolactic fermentation. After 5 months wines were bottled in 750 ml bottles sealed with corks.

1999 trials: A total of 500 kg of grapes was destemmed, crushed, and divided into 4 homogeneous 125 kg batches of must; 20 mg·l$^{-1}$ of $SO_2$ were added. All batches were inoculated with yeast (20 g dry wt·hl$^{-1}$) as described above and punched down three times a day during active fermentation. The following maceration treatments were carried out:

Variant 1A: maceration temperature 25 ºC, pressing after 6 d, pressing when 60 % of the available sugars were fermented; Variant 2A: maceration temperature 25 ºC, pressing after 11 d at dryness; Variant 3A: maceration temperature 25 ºC, pressing after 19 d; Variant 4A: maceration temperature: day 1 to 17 at 25 ºC; day 17 to 19 at 35 ºC; pressing after 19 d.

After maceration, each lot was pressed as in 1998 and variant 1A was allowed to ferment completely. Each wine was divided into two equal parts. 50 mg·l$^{-1}$ $SO_2$ were added to the first part in order to prevent malolactic fermentation; malolactic bacteria (2 g dry wt·hl$^{-1}$) were added to the second part of each lot. Malolactic fermentation was carried out at 20 ºC. At the end of malolactic fermentation 50 mg·l$^{-1}$ $SO_2$ were added. The malolactic fermentation variants were numbered: 1B, 2B, 3B, 4B. All wines were stored in 30 l stainless steel tanks at 15 ºC until analysis in February 2000.

Chemicals: All chromatographic solvents (methylene chloride, methanol, formic acid) were of HPLC grade (Fisher Scientific, Santa Clara, CA, USA). Heptanesulfonic acid (HPLC grade) was purchased from Alltech (Deerfield, IL, USA), (-)-epicatechin from Sigma (St. Louis, MO, USA), (+)-catechin from Fluka (Switzerland), Folin-Ciocalteu reagent and vanillin from Merck (Germany). Cacao bean proanthocyanidin extract was prepared as described by RIGAUD *et al.* (1993).

Grape extracts: Grape extracts were prepared according to MATTIVI *et al.* (2001). The skins and the seeds of 200 g of berries (vintage 1999) were carefully separated, then transferred into a 500 ml flask containing 250 ml of a synthetic wine-like medium (12 vol% ethanol, pH 3.2, 50 mg·l$^{-1}$ $SO_2$), and were extracted for 5 d at 30 °C, shaking the flask twice a day. After decanting the clear liquid, the residual solids were pressed by hand and the total liquid was brought to 250 ml. This was transferred into a glass bottle and stored at 4 °C until analysis.

Sample preparation for HPLC analysis: Wine samples were prepared following the method of KENNEDY and WATERHOUSE (2000), except that no caffeine was added to improve recovery of skin tannins since no improvement by adding caffeine was observed.

HPLC method: The proanthocyanidins (P) of wine were determined by integrating two areas of the chromatogram at 280 nm separately, the values corresponding to proanthocyanidins from 2 to 4 units (LMWP) and to proanthocyanidins formed by 5 or higher units (HMWP), as described previously (VRHOVSEK *et al.* 2001). A cacao bean proanthocyanidin extract was injected at the beginning and at the end of each sequence (about 8 wine samples) in order to calibrate the retention times of different oligomers. Quantitative data were calculated on the basis of a calibration curve, with the external standard method, and expressed as epicatechin equivalents, mg·l$^{-1}$. P is the sum of LMWP and HMWP.

Spectrophotometric methods: Spectrophotometric methods (DI STEFANO *et al.* 1989) were carried out under optimized conditions as described by RIGO *et al.* (2000).

Total phenols (FC): Total phenols were assessed by the reduction of phosphotungstic-phosphomolybdic acids (Folin-Ciocalteu reagent) to blue pigments by phenols in alkaline solution. Concentrations were determined by means of a calibration curve as (+)-catechin, mg·l$^{-1}$.

Proanthocyanidins (PROC): The proanthocyanidins were evaluated by transformation into cyanidin (DI STEFANO *et al.* 1989) and expressed by means of a calibration curve with cyanidin chloride. PROC is more correlated to large proanthocyanidins.

Index of vanillin (VAN): The catechins and proanthocyanidins reactive to vanillin were analyzed according to the optimised and controlled vanillin-HCl method of BROADHURST and JONES (1978), following the conditions described by DI STEFANO *et al.* (1989). Concentrations were calculated as (+)-catechin (mg·l$^{-1}$) by means of a calibration curve. The VAN index is more sensitive to monomers (catechins) and small proanthocyanidins.

Total anthocyanins (AT): The total anthocyanins were directly quantified on the basis of the maximal absorbance in the visible range (536-540 nm). The wine pigment content (mg·l$^{-1}$ ) was calculated assuming an average molar absorption of the mixture of anthocyanins to equal 18,800 M$^{-1}$ cm$^{-1}$ (DI STEFANO *et al.* 1989).

Color intensity and nuance (hue): The color characteristics, intensity (A420+A520) and nuance (ATAN (A520-A420)), have been measured directly on the basis of absorption at 420 and 520 nm, registering the measurement in a 1 mm optical path cell and multiplying the results by 10, since measure is conventionally referred to the optical path of 10 mm.

## Results and Discussion

Preliminary results (1998 trials): Preliminary results had indicated that cv. Blaufränkisch grapes (mash) require a long maceration time to obtain a maximum extraction of proanthocyanidins. The results clearly indicate a continuous increase of proanthocyanidins within 15-19 d, while a longer contact led to some losses. The extraction of the LMWP and HMWP increased up to day 19 (Figure).

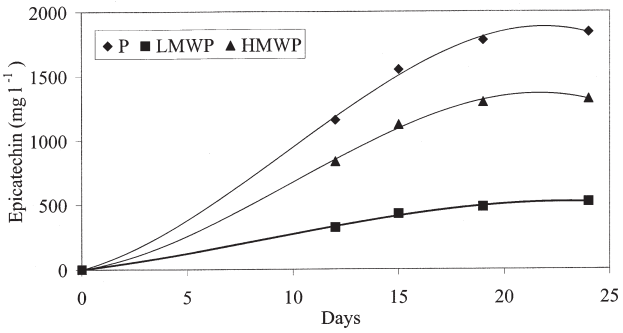


Figure: Extraction of different classes of proanthocyanidins during maceration, vintage 1998. P: total proanthocyanidins; LMWP, HMWP: low resp. high molecular weight proanthocyanidins.

1999 trials. Grapes: The prolonged maceration time, which was necessary to reach the maximal extraction in the 1998 trials, suggested an important contribution of grape seeds to this wine. Compartmentation of the phenolics in the skin and seeds was estimated by a separate extraction of skins and seeds from a sample of the 1999 grape berries. The extraction was carried out with a synthetic medium designed to reproduce wine, to extract only the wine soluble (*i.e.* "extractable") phenolics and not the higher polymers observed in strong solvent extracts, suitable for the study of the total proanthocyanidins (such as the mixture acetone/$H_2O$: 70/30). Tab. 1 clearly demonstrates that the extractable phenolics and both, the LMWP and the HMWP are divided nearly equally between skins and seeds, LMWP being slightly higher in the seeds, HMWP being higher in the skin. The higher values of the VAN parameter observed in the seeds (Tab. 1) could be due to the presence of high amounts of monomers in the seeds, but this hypothesis has to be confirmed by further HPLC investigation. The values measured in grapes can be used to evaluate the approximate yield of extraction during vinification, whereas the fermentative process, the yeast enzymes (*e.g.* β-glucosidase), and the absorption on the fermentation lees could cause losses and transformation of anthocyanins and other phenolics (Boulton *et al.* 1996).

Effect of maceration time: Extended maceration increases all proanthocyanidins (LMWP, HMWP) (Tab. 2). In series A the increase between 1A and 3A was larger for HMWP (34.2 %) compared to LMWP (21.6 %), but it must be taken into consideration that flavan 3-ol monomers, with high concentrations in young wines are not included in LMWP. This is confirmed by results of spectrophotometric measurements, since the increase of VAN is greater than the increase of PROC (Tab. 3). The VAN index is more sensitive to monomers (catechins) and small pro-anthocyanidins, while PROC is more correlated to large pro-anthocyanidins.

Different maceration times did not significantly influence the intensity and hue of color (Tab. 3), which agrees with results of Kovac *et al.* (1992). AT showed a decrease after 19 d of maceration (3A), which is possibly due to the adsorption of anthocyanins on solid parts of the grapes and lees during prolonged maceration, after reaching their maximal value during the early phases of maceration.

Effect of wine heating: In variant 4, the wine was warmed and kept at about 35 ºC for 48 h at the end of maceration, with the primary aim to obtain more color. The treatment resulted in a significant increase of all polyphenols. Spectrophotometric analyses showed an important increase in the amounts of AT (24 %), PROC (20 %) and VAN (15 %) in variant 4A compared to 3A, which was macerated for the same time, but without raising T in the last 48 h (Tab. 3). The results for VAN are in agreement with the data reported by Oszmianski *et al.* (1986) who reported that the effect of high maceration temperature (35 ºC) seems to be particularly important for the release of low molecular weight phenolics.

Heating not only increased the total anthocyanin content, but also led to an increase of color intensity (Tab. 3). The values measured in skin and seed extracts were used to estimate the theoretical (maximal) concentration which can be expected in wine, considering an approximate extraction of 0.7 l of wine per kg of grapes (*i.e.* it can be obtained by dividing the amounts reported in Tab. 1 by 0.7). Extraction of anthocyanins in the wine 4A reached 52.5 %. The results obtained with NP-HPLC analysis showed that HMWP increased more than LMWP (Tab. 2). All parameters directly linked to the proanthocyanidins (PROC, HMWP, LMWP) indicate that this winemaking procedure gives a very high yield, *i.e.* between 78.3 and 89.7 % of the maximal concentration estimated on the basis of the grape analysis (Tab. 1).

The total extraction of phenols (FC) was estimated to be 71.4 %. The VAN parameter could possibly underestimate the extraction yield of catechins and proanthocyanidins (59.8 %) since it is sensitive to the polymerization processes that may have occurred during winemaking and storage.

Effect of malolactic fermentation: In all cases malolactic fermentation led to a slightly lower concentration of anthocyanins (AT) (Tab. 3). The difference was significant only for the 4A-B variants. This effect was

Table 1

Compartmentation of extractable polyphenols in skin and seed extracts (synthetic wine-like media), vintage 1999. AT: total anthocyanins; FC: total phenols; VAN: index of vanillin; PROC: proanthocyanidins; LMWP, HMWP, P: see Figure

| | AT | FC[a] | VAN[a] | PROC[b] | LMWP[c] | HMWP[c] | P[c] | LMWP/HMWP |
|---|---|---|---|---|---|---|---|---|
| Skins (g kg$^{-1}$ grape) | 1.07 | 1.12 | 0.42 | 1.05 | 0.37 | 1.12 | 1.49 | 0.33 |
| Seeds (g kg$^{-1}$ grape) | 0.00 | 1.04 | 1.38 | 0.93 | 0.40 | 1.05 | 1.44 | 0.38 |
| Grape, total (g kg$^{-1}$ grape) | 1.07 | 2.16 | 1.80 | 1.98 | 0.77 | 2.17 | 2.93 | |

[a] equivalent of (+) catechin; [b] equivalent of cyanidin; [c] equivalent of (-) epicatechin.

Table 2

Effect of maceration time (1A-3A), must heating (4A) and malolactic fermentation (1B-4B) on the concentration of LMWP and HMWP, vintage 1999. P, LMWP, HMWP: see Figure

| Variant | Maceration (d) | P[a] | LMWP[a] | HMWP[a] | LMWP/ HMWP |
|---|---|---|---|---|---|
| 1 A | 6 | 2.33 | 0.73 | 1.60 | 0.46 |
| 2 A | 11 | 2.71 | 0.83 | 1.88 | 0.44 |
| 3 A | 19 | 3.04 | 0.89 | 2.15 | 0.41 |
| 4 A | 19 | 3.41 | 0.98 | 2.43 | 0.40 |
| 1 B | 6 | 2.38 | 0.74 | 1.64 | 0.45 |
| 2 B | 11 | 2.46 | 0.73 | 1.73 | 0.42 |
| 3 B | 19 | 2.81 | 0.82 | 1.99 | 0.41 |
| 4 B | 19 | 3.32 | 0.91 | 2.32 | 0.39 |

[a] equivalent of (-) epicatechin.

expected since malolactic bacteria have ß-glucosidase activity (DITTRICH 1987) that would hydrolyze the anthocyanins to unstable anthocyanidins. The intensity of wine color is also lower in wines subjected to malolactic fermentation (Tab. 3). Results of hue show that wines produced without malolactic fermentation are slightly more purple due to a lower pH and higher percentages of flavilium form (Tab. 3). Malolactic fermentation does not affect VAN and PROC, except for 2A-B. On the other hand NP-HPLC analysis shows a slight decrease in the concentration of P, HMWP and LMWP, except between 1A and 1B. This decrease should not be due to a different time course of reactions like direct condensation and acid hydrolysis of proantho-cyanidins at different pH, since the products of both reactions are still absorbing at 280 nm and included in the NP-HPLC measure; but they might interact with cellular membranes of bacteria; this speculative hypothesis has to be evaluated by further experiments.

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Table 3

Effect of maceration time (1A-3A), must heating (4A) and malolactic fermentation (1B-4B) on color characteristics and concentrations of different classes of polyphenols, vintage 1999. AT, FC, VAN, PROC: see Tab. 1

| Variant | Maceration (d) | pH | Color intensity | Hue (degree) | AT (g $l^{-1}$) | FC (g $l^{-1}$)[a] | VAN (g $l^{-1}$)[a] | PROC (g $l^{-1}$)[b] |
|---|---|---|---|---|---|---|---|---|
| 1 A | 6 | 3.27 | 12.5 | 77.0 | 0.76 | 1.73 | 0.96 | 1.61 |
| 2 A | 11 | 3.25 | 13.3 | 77.8 | 0.78 | 1.85 | 1.20 | 1.89 |
| 3 A | 19 | 3.42 | 12.7 | 75.0 | 0.65 | 2.06 | 1.34 | 1.95 |
| 4 A | 19 | 3.28 | 15.3 | 78.7 | 0.80 | 2.21 | 1.54 | 2.33 |
| 1 B | 6 | 3.41 | 9.5 | 67.4 | 0.76 | 1.72 | 0.99 | 1.70 |
| 2 B | 11 | 3.40 | 9.8 | 68.6 | 0.78 | 1.76 | 1.03 | 1.82 |
| 3 B | 19 | 3.46 | 10.0 | 67.4 | 0.62 | 1.87 | 1.32 | 1.92 |
| 4 B | 19 | 3.40 | 12.6 | 73.5 | 0.73 | 2.20 | 1.52 | 2.34 |

[a] equivalent of (+) catechin; [b] equivalent of cyanidin.

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