Effect of cold maceration, stem return, re-heating and mannoprotein addition on color stabilization of 'Pinot noir' wines

CARLOS DE LA BARRA[1], KONRAD HACKL [2], KLAUS DÜRRSCHMID[1], REINHARD EDER [3] und KLAUS DIETER KULBE[1]

[1] Department Lebensmittelwissenschaften und -technologie, Universität für Bodenkultur Wien
   A-1190 Wien, Muthgasse 18
   E-mail: Carlos.Delabarra@chello.at

[2] Niederösterreichische Landeslandwirtschaftskammer
   A-3500 Krems, Sigleithenstraße 50

[3] Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau
   A-3400 Klosterneuburg, Wiener Straße 74

Large-scale (210 l) fermentation with cold maceration of 'Pinot noir' musts from the Krems Valley (Austria) showed that the procedures used - stem return, re-heating after fermentation, a combination of both methods, and addition of mannoprotein - have a pronounced influence on the extraction and stabilization of the color. All wines had a similar alcohol level (~13.7 %vol) but significant differences in the level of malic acid could be observed (e.g. stem-return + 148 % compared to control). The use of mannoprotein had a positive influence on the level of stabilized malvidine-3-glucoside (+ 41.3 % compared to control). The loss of malvidine-3-glucoside was the largest at the final stage of malolactic fermentation. The color intensity remained essentially unchanged during processing, the variant with combination of stem-return and re-heating showed the lowest level (-14.4 % c. to c.), the mannoprotein variant the highest values (+ 22.7 % c. to c.). The tannin content showed a difference of up to 19.3 % between the stem-return variant and the control. Sensory evaluation with triangular test showed that the wines could be easily distinguished. Descriptive analysis was used to produce a sensory profile for each variant and to estimate correlation between the sensory characteristics and analytical data. It was found out that the examiners differentiated the wines essentially on the basis of color.
Keywords: red wine, 'Pinot noir', color stabilisation, malvidine-3-glucoside, mannoprotein, stem-return, re-heating

Auswirkung von Kaltmazeration, Kammrückgabe, Nacherwärmung und Mannoproteinzugabe auf die Farbstabilität von Weinen der Sorte 'Blauer Burgunder'. *Bei Gärversuchen mit Kaltmazeration im Großmaßstab (210 l) bei der Sorte 'Blauer Burgunder' aus dem Kremstal (Österreich) zeigte sich, dass die angewandten Verfahren - Kammrückgabe, Nacherwärmung nach der Gärung, Kombination aus Kammrückgabe und Nacherwärmung, Mannoprotein-Zugabe - einen deutlichen Einfluss auf die Extraktion und die Stabilisierung der Farbe hatten. Alle Weine hatten vergleichbare Alkoholgehalte (~13,7 %vol), aber beim Gehalt an Äpfelsäure konnten deutliche Unterschiede festgestellt werden (z.B. Kammrückgabe + 148 % gegenüber Kontrolle). Die stärkste Abnahme von Malvidin-3-glucosid fand in der Endphase des biologischen Säureabbaus statt. Besonders durch die Mannoproteinzugabe konnte der Gehalt an stabilisiertem Malvidin-3-glucosid erhöht werden (+41,3 % g. K.). Die Farbtiefe wurde durch die verschiedenen Vinifizierungstechniken beeinflusst, der Kombinationsansatz (Kammrückgabe und Nacherwärmung) hatte eine um 14,4 % geringere, und die Variante mit Mannoproteinzugabe eine um 22,7 % höhere Farbintensität als die Kontrolle. Die Variante mit Kammrückgabe wies den höchsten Tanningehalt auf (+19,3 % g. K.). Sensorische Dreiecksprüfungen ergaben, dass die Weine deutlich zu unterscheiden waren. Mit einer deskriptiven Analyse konnte für jede Variante ein sensorisches Profil erstellt und die Korrelation zwischen sensorischen Merkmalen und analyti-*

schen Daten ermittelt werden. Dabei zeigte sich, dass die Weine von den Prüfern im Wesentlichen anhand der Farbe unterschieden wurden.

Schlagwörter: Rotwein, 'Pinot noir', Farbstabilisierung, Malvidin-3-glucosid, Mannoprotein, Kammrückgabe, Nacherwärmung

Les effets de la macération froide, du rajout des rafles, d'un réchauffement ultérieur et de l'addition de mannoprotéine sur la stabilité de la couleur des vins du cépage 'Blauer Burgunder'. *Il s'est avéré au cours d'essais de fermentation avec macération froide à grande échelle (210 l) effectués avec le cépage 'Blauer Burgunder' du Kremstal (Autriche) que les procédures appliquées - rajout des rafles, réchauffement après la fermentation, combinaison entre rajout des rafles et réchauffement ultérieur, ajout de mannoprotéine - exerçaient une influence importante sur léxtraction et la stabilisation de la couleur. Tous les vins présentaient des teneurs en alcool comparables (~13,7 %vol), mais on a pu constater d'importantes différences en ce qui concerne la teneur en acide malique (p. ex. rajout des rafles + 148 % vis-à-vis du contrôle). La réduction la plus forte de la malvidine-3-glucoside avait lieu au cours de la phase finale de la dégradation biologique de l'acide. La teneur en malvidine-3-glucoside stabilisée a pu être augmentée notamment par l'ajout de la mannoprotéine (+41,3 % vis-à-vis du contrôle). L'intensité de la couleur a été influencée par les différentes techniques de vinification, la variante combinée (rajout des rafles et réchauffement ultérieur) présentait une intensité de couleur inférieure de 14,4 % et la variante avec ajout de mannoprotéine présentait une intensité de couleur de 22,7 % plus élevée que le contrôle. La variante avec rajout des rafles présentait la teneur en tannin la plus élevée (+19,3 % vis-à-vis du contrôle). Il a résulté des essais triangulaires sensoriels que les vins se distinguaient sensiblement. Il a été possible d'établir un profil sensoriel pour chaque variété à l'aide d'une analyse descriptive et de déterminer la corrélation entre les caractéristiques sensorielles et les données analytiques. Il s'est avéré à cette occasion que les testeurs différenciaient essentiellement les vins sur la base de leur couleur.*

Mots clés: vin rouge, 'Pinot noir', stabilisation de la couleur, malvidine-3-glucoside, mannoprotéine, rajout des rafles, réchauffement ultérieur

The cultivar 'Pinot noir', one of the most popular grape cultivars worldwide, has its origin in Burgundy in France, and develops particularly well in cool climates (CLIFF and DEVER, 1996). It is estimated that approximately 45 000 hectares are cultivated with this cultivar throughout the world. In Austria approximately 140 hectares of 'Pinot noir' are planted, with a significant increase in the last years (SCHMID, 2002; ÖWM, 2003). The cultivar is described as being problematic (GERBAUX et al., 1998) and there are numerous different clones of this vine (BESSIS et al., 1998; BERNARD, 1998). The character of 'Pinot noir' wine varies more greatly as a function of soil, climate and vinification than do other cultivars (ROBINSON, 2003). Polyphenol compounds are primarily responsible for this so-called varietal characteristic (AUBRY et al., 1999). Their composition and concentration are decisive for the typification and style of the wines. Wines can be examined, in regard to phenol management, from the vineyard to the bottle (ZOECKLEIN et al., 1995). In addition, these phenols are important oxygen reservoirs and function as a substrate for browning reactions. There are a large number of phenolic compounds in wine which are derivates of hydroxy-benzenes. In the grapes, and later in the wine, one can differentiate between non-flavonoid and flavonoid phenols. The substances which produce color in wine are complexes of phenolic molecules, whereby, the most common are formed from anthocyanins and tannins (proanthocyanidines) (PEYRON, 1998). In 'Pinot noir' the anthocyanins delphinidine, cyanidine, petunidine and malvidine are present only as 3-monoglucosides (GAO et al., 1997; EDER et al., 1994).

In wine, however, the concentration of these molecules is lower than in the fruit. As a mean value, literature specifies a maximum value of 60 % after removing the grapes from the stems and crushing (MARGALIT, 1997). The return of the mature (lignified) stems after destemming is a traditional method, which is rarely used today in vinification. The presence of stems alters various factors: the polyphenol quantity increases giving the wine a spicy character. The tannins in the stems differ from those in the grape skins; the former show a marked tendency towards being more adstringent and rougher (ZOECKLEIN et al., 1995; RIBÉREAU-GAYON and GLORIES, 1986). Temperature has a fundamental influence on the extraction of polyphenols. Low temperatures during fermentation are reflected in a fruity character of the wine, whereas higher temperatures result in a better diffusion of components such as anthocyanins and phenols from the grape skins. After alcoholic fermentation,

the appropriate temperature also has a major influence. Within the scope of these experiments, it can be expected that a post-crushing resting period of approximately 42 hours at a temperature of 40 °C would benefit the formation of complexes between anthocyanins and tannins (FEUILLAT, 1999). A pre-fermentative cold maceration at a controlled temperature of 6 °C is carried out in order to produce better aroma complexity and increased color extraction and stabilization (SCHÖDL, 2002). In the absence of alcohol, a complex formation between soluble anthocyanins and the phenols, already present in the solution, can take place, which stabilizes the color. This process - an aerobic process - should take place at a low temperature in order to prevent spontaneous fermentation. ZOECKLEIN (1994) proved that a cold maceration period of at least 48 hours results in a deeper color and an increase in color intensity. The extraction maximum for anthocyanins is reached approximately halfway through fermentation (ZOECKLEIN et al., 1995). If the must is allowed to rest longer, the complex formation of the tannin structure of the wine and its color stabilization is favored. Tannins which result from this prolonged contact with the must appear to stabilize the anthocyanins by building polymer complexes (MARGALIT, 1997).

Considering the changes in consumer behavior and technical possibilities, which allow winegrowers to offer better quality, wine production must be optimized in respect to the characteristics of the individual cultivars. In the case of 'Pinot noir', the focus is clearly on its color. This characteristic is one of the decisive reasons for purchase. The consumer connects the good quality of a (red) wine with its strong color, whereby 'Pinot noir' is at a disadvantage compared to other more strongly colored cultivars. This makes it particularly important that this primary characteristic is improved to the greatest possible extent. For this reason, this paper discusses attempts to increase and stabilize the color of 'Pinot noir' by polymer formation between anthocyanins and polyphenols.

Material and methods

Must (210 l) from destemmed 'Pinot noir' grapes from the Krems Valley in Lower Austria was fermented in five variants with one repetition each (see Table 1). These were: a control variant (I), one with returned stems (II), one with post-fermentation re-heating (III), a combination of the last two (IV), and a variant (V) with the addition of mannoproteins.

The grapes were removed from the stems and crushed gently, put in 300 l fermentation vessels (measurements 120 x 100 x 58 cms.), divided into equal amounts (16 kg $\pm$ 0.2 kg of must). The must was stored in a cooling chamber at +6 °C for seven days and the cap submerged twice daily. For the variants with returned stems (II and IV), the stems of the total grape mass were weighed and the adequate amount (20 % of the weight = 3.2 kg) of the original stems returned to the must. To variant V 30 g/hl mannoprotein (OptiRed, Lallemand). was added. After the cold maceration (7 days, 6°C), the alcoholic fermentation was started with the addition of ~20 g/hl of *Saccharomyces cerevisiae* (BM45, Lalvin) at an initial temperature of 17.5 °C. After fermentation, the young wine rested for 14 days at a temperature of approximately 20 °C. During the 'end maceration' of

Table 1:
Overview of the variants

Method	Variant I Control 1 (PN 01) 2 (PN 02)	Variant II Stem return 3 (PN 03) 4 (PN 04)	Variant III Re-heating 5 (PN 05) 6 (PN 06)	Variant IV Comb. II + III 7 (PN 07) 8 (PN 08)	Variant V Mannoprotein 9 (PN 09) 10 (PN 10)
Cold maceration (5 °C, 7 days)	+	+	+	+	+
Enzyme addition (4 g/hl)	+	+	+	+	+
Stem return (20 % weight)	-	+	-	+	-
Re-heating (40 °C, 42 hours)	-	-	+	+	-
Mannoprotein (30 g/hl)	-	-	-	-	+
Yeast addition (BM45 Strain)	+	+	+	+	+
Must resting period (20 °C, 14 days)	+	+	+	+	+
Bacteria addition (0,5 g/l; post ferment.)	+	+	+	+	+
Storage (14 °C, cellar)	+	+	+	+	+

variants III and IV, the completely fermented must was re-heated to approximately 45 °C for 42 hours using a heat exchanger (Klinger, Langenlois). The wines underwent malolactic fermentation in glass demijohns using an *Oenococcus oeni* starter culture (0.5 g/l bacteria culture (LQ54, Lalvin). Then the SO_2 content was adjusted to 50 mg/l. Over a period of 244 days usually once a week samples were taken (250 ml wine).

Analyses

All analyses were carried out at the Departement of Food Sciences and Technology, BOKU-University of Natural Resources and Applied Life Sciences,Vienna. A HPLC method (Eder et al., 1990) in an adapted form was used for the classification of anthocyanins with external standards (malvidine-3-glucoside, Extra-synthese Cie., France). The samples were centrifuged at 2000 rpm for two minutes and then separated in a reversed phase column (LC-18-DB, Supelcosil, Supelco) with a 5 mM phosphate buffer, pH = 1,8 (A) and HPLC-grade methanol (Roth) as an eluent (B). The gradient was made up as follows: start with 25 % B, increase to 30 % B in 30 minutes. A leap to 100 % B for 10 minutes to guarantee complete elution; following this, equilibration to 25 % B for 20 minutes. The program ended after 60 minutes. The flow rate was 0.8 ml/min, the pressure ~88 bar. The temperature was set at 40 °C (nominal), the sample volume was 10 µl. The color values (color intensity and color tone) were determined using spectrometry in both the visible and UV areas. The absorption of the samples, diluted 1:10 with de-ionized water and adjusted to the wine pH-value, was measured in quartz cuvettes at 420 and 520 nm. The intensity of the wine color (I) is seen as the sum of the measurements at 420 and 520 nm. On the other hand, the color hue (tone, H) is calculated as the quotient of the measurements at 420 to 520 nm. Using the Folin-Ciocalteu reagent the concentration of total phenols was analysed at an absorption wavelength of 765 nm. The tannin calculations were made with a spectrometry in the visible area (Zoecklein et al., 1995), with the only alteration being that quercitine was used as the standard for the calibration curve instead of gallic acid. After 244 days, the wines were submitted to two sensory investigations, a triangular control and a descriptive analysis. These examinations were carried out in the Sensory Laboratory of the Institute for Food Technology. It has eight test cubicles with white light (8 Philips mercury discharge lamps TLD 18W/33, in

each) and computer terminals for recording the responses of the panel members. The location with the test cubicles has an overpressure of 1400 m^3/h supply air against 1170 m^3/h (this impedes the intrusion of disturbing odors). The eight trained, professional wine tasters on the examination panel were given the samples in norm glasses in accordance with ISO 3095. The programs Excel XP, sensory software PSA/System 3 and SensTools were used for the registration and evaluation of all data and StatGraphics 4.0 was implemented for the statistical evaluation.

Results and discussion

Chemical analysis of the wines showed that all wines had the same alcohol level (~13.7 %vol). Content of malic acid was analysed enzymatically, only the variant with stem-return had a distinct higher value of malic acid, the progress of malolactic fermentation was controlled by thinlayer chromatography and showed no signficant differences. Figure 1 depicts the time course of the calculated color values for variant 10 (mannoprotein
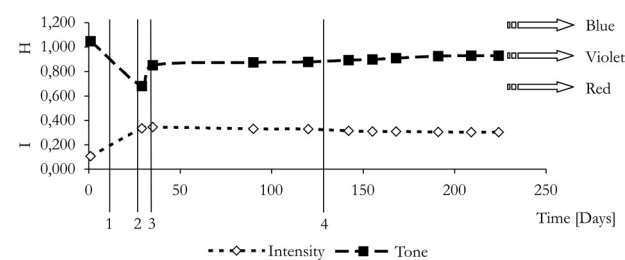
Figure 1: Time course of the color values (intensity = I and tone = H) for wine variant 10 (addition of mannoprotein). The points-of-time 1 and 2 show the beginning and completion of alcoholic fermentation, respectively; points 3 and 4 show the beginning and completion of the malolactic fermentation (the same applies for all Figures in this paper).

addition). This course is typical of all variants. It makes apparent the behavior of color tones and intensity during the individual phases of the experiment.

The time course of the total phenol content is depicted in Figure 2 for wine variant 10 (mannoprotein addition). The course is typical for all courses. It was possible to calculate polymerized phenols from the data used. Using this it can be discussed in detail that, with the appearance of tannins in solution (during fermenta-

tion) the polymerization of phenol compounds initially showed a constant increase. After the beginning of the malolactic fermentation, the process decreased drastically, setting a lesser, but steady, re-polymerization into action. It is not possible to make any more detailed statement concerning the nature of these polymerization activities, in the sense of the actual formation of
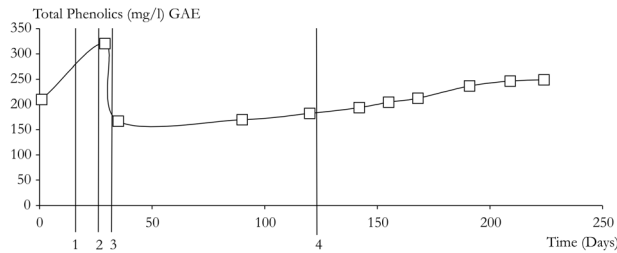

Figure 2: Development of total phenol content over time for variant 10

cross networks of polyphenols, in this paper.

The typical time courses of the tannin content, of two variants (variants 3 + 4 = stem return; variants 9 + 10 = mannoprotein addition) are compared in Figure 3. This representation helps in the understanding of the preceding conclusions on the polymerization of phenols. Tannins (Fig. 3) show a completely different course than the phenols (Fig 2).

The total time course of the content of malvidine-3-glucoside in three variants (2 = control; 9 + 10 = mannoprotein) can be seen in Figure 4. This makes it clear that addition of mannoprotein (9 and 10) causes a flattening of the curve, at the end of the time under obser-
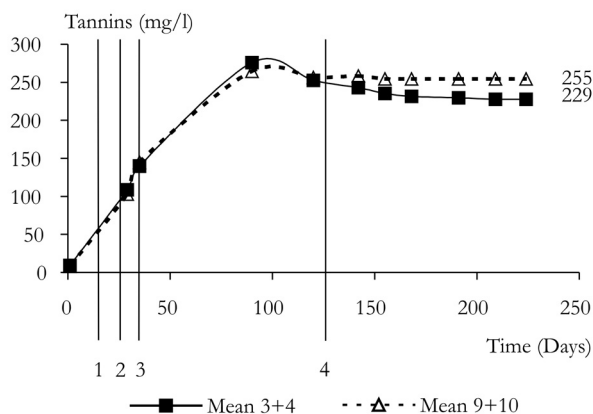

Figure 3: Comparison of the time course of the tannin content between the variants with mannoprotein (variants 9 + 10) and stem return (3 + 4)
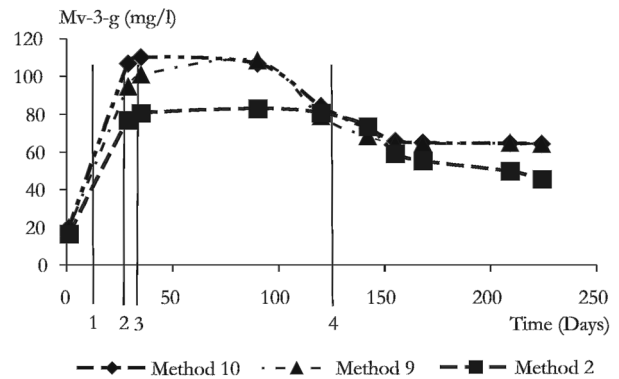

Figure 4: Time course of the amount of malvidine-3-glucoside.

vation, compared to the control procedure. This indicates a higher stabilization of malvidine-3-glucoside.

The results of the sensory triangular test are summarized in Table 2. The wine tasters were able to differentiate between the different wines in a statistically significant manner. Based on these results, a descriptive analysis was carried out to investigate these differences.

A total depiction of the descriptive analysis, along with the analytical data for the malvidine-3-glucoside, is given in Figure 5 in the form of a spider plot. It is appa-

Table 2:
Summary of the results of the triangular test

Examiner	Number of comparisons	Correct	False
1	24	23	1
2	24	20	4
3	24	21	3
4	24	18	6
5	24	20	4
6	24	23	1
7	24	21	3
8	24	24	0

rent that addition of mannoprotein displayed positive characteristics to the wine more clearly than all the other variants. For the control, it was only possible to depict a much less marked sensory profile, based on the data of the descriptive analysis. Other variants are located between the control procedures and the methods with mannoprotein. The single exception is the variant with re-heating for the characteristic „violet".

A principal component analysis (PCA) was made using the data from the sensory analysis. Figure 6 was produced on the basis of this PCA. It is apparent that the examiners placed the wines according to their appearance.
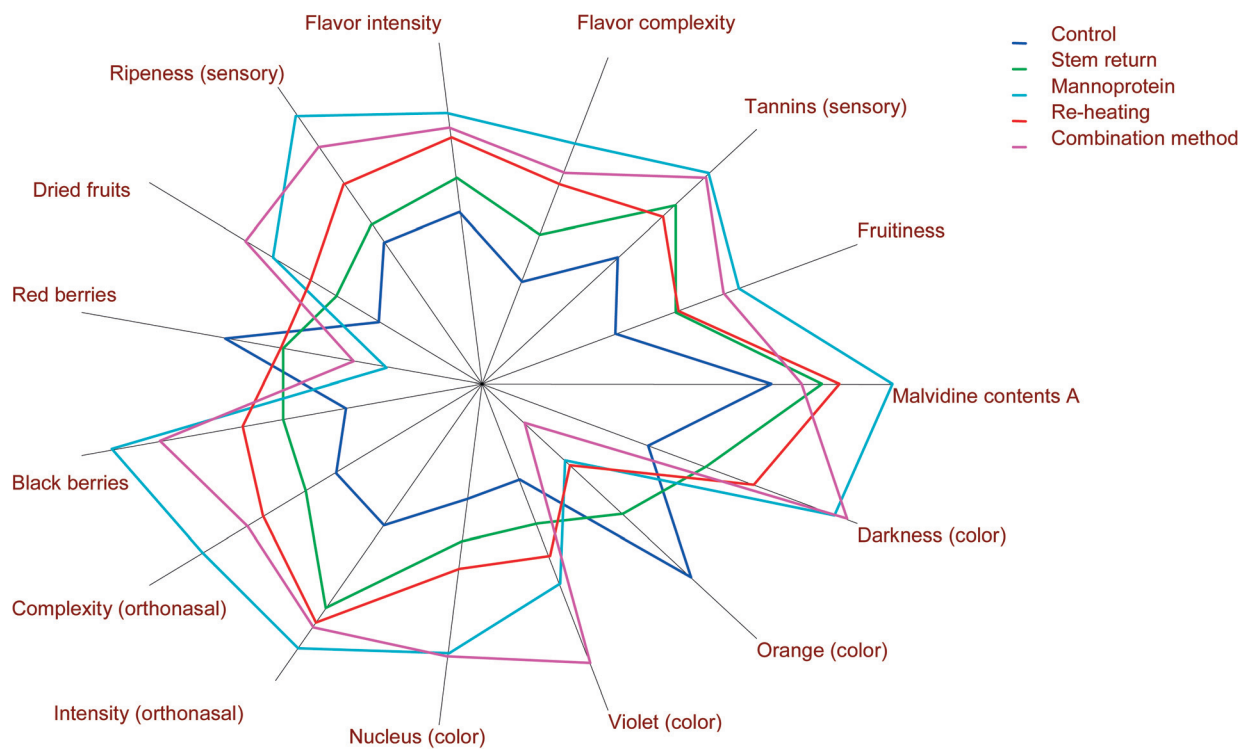
Figure 5: Representation of the sensory profiles of the various wines, based on the data from the descriptive analysis. Mean values are shown. „A" denotes analytical data.
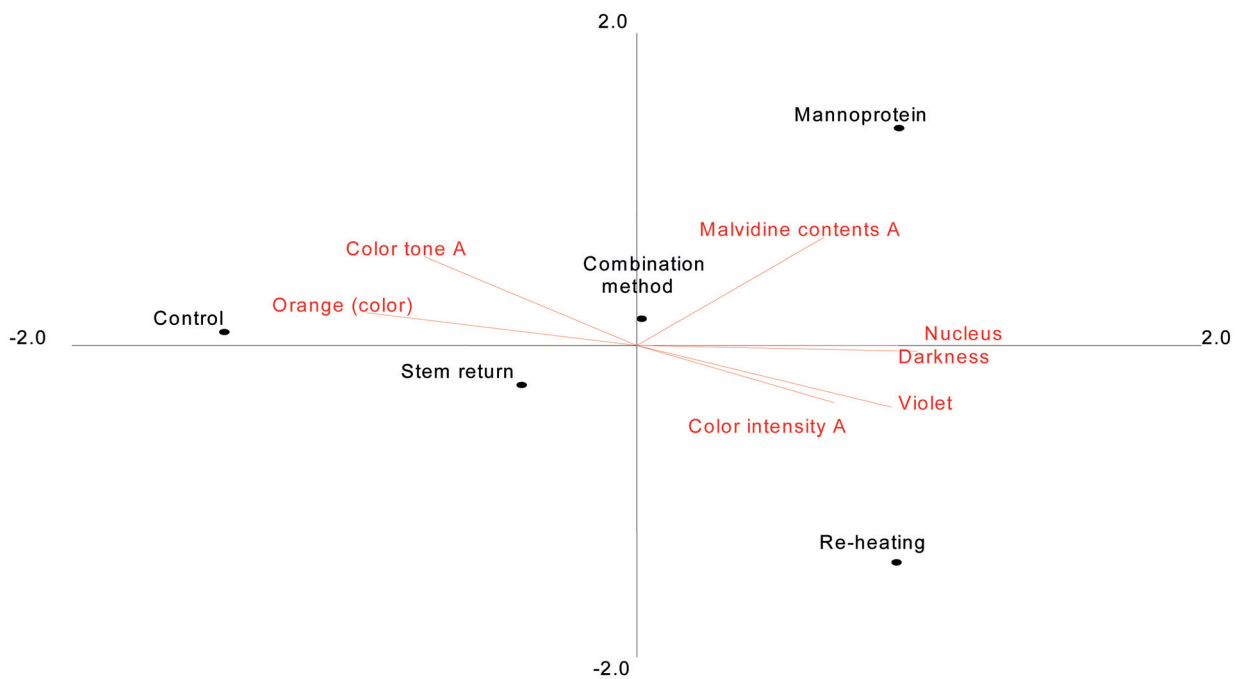

Figure 6: PCA plot for the appearance of the wines. „A" denotes analytical data

Table 3:
Summary of chemical and sensory results for all wines

	Variant I Control (Standard)	Variant II Stem return	Variant III Re-heating	Variant IV Comb. II + III	Variant V Mannoprotein
Color intensity $I = (A_{420} + A_{520})$	I = 0,247	+ 12,6%	+ 13%	+ 19%	+ 22,7%
Malvidine-3-glucoside (analytical)	45,5 mg/L	+ 9,6%	+ 10,7%	+22,4%	+ 41,3%
Tannins (analytical)	214 mg/L	+ 19,3%	- 0,5%	- 11,1%	+ 7,2%
Overall sensory profile	Highest values on negative aspects such as "red berries" and "orange color".	Sensory profile close to the standard method. No positive increasements.	Same as for variant II, difficulties to be evaluated by the sensory panel.	Close to the best evaluated, at less analytical values. Good appearance.	Best profile in the tasting. Best appearance, and best over-all wine.
Color (sensory)	Light color with "bad tones".	Hence a little bit darker, similar to control.	Appearance in the middle field. Good color tone.	Good appearance, dark color with best violet tone.	Best over-all appearance, best color tone.
Tannins (sensory)	Less ripe and more rough tannins.	Similar to control, with even more (rough) tannins.	Less rough tannins as variant II.	Smoother tannins, good quantity (sensory).	Smooth tannins, best at the tasting.
Complexity (sensory)	Less complexity, ortho- as well as retronasal.	Presented difficulties for the panel to be evaluated. Middle field.	Similar to variant II, but better flavor, and more intensity.	More complex on the palate, in the nose closer to variant III.	Best complexity, ortho- as well as retronasal.

In Figure 6 it is made clear that the panelists had some difficulties on differentiating the variants with stem return, and the combination of stem return and re-heating. The sensory panel placed these two variants close to the middle of the evaluated spectrum of wines.

The next table (Table 3) shows a summary of chemical and sensory results achieved with this experimental design.

On both figures of the sensory evaluation (Figure 5 and 6) it is observable that a variant with better analytical values such as color-intensity and color-tone, as well as a higher content of stabilized malvidine-3-glucoside, showed a better profile in the sensory evaluation. This is an indicator for the fact that wines with better color are frequently the wines that are better evaluated in a sensory analysis. Therefore, it was possible to establish a positive correlation between the chemical-analytical data (marked with an „A" on the figures with data from the sensory analysis) and the sensory evaluations made to these wines. Wines made with the stem return method were by far the wines evaluated the closest to the control wines (standard vinification as described). The wines made by the re-heating method, were better evaluated in some characteristics (as shown in Figure 5 and 6), while being similar to wines made by stem re-

turn in some characteristics such as orthonasal complexity. Even though the appearance of the wines made with the re-heating method were close to the wines made with mannoprotein, the sensory panel was able to distinguish between the different approaches, as said before. Finally, it is possible to affirm that the wines treated with mannoprotein, while giving a higher content of stabilized malvidine-3-glucoside thus showing a higher color, were the wines with the best overall sensory profile, despite the fact that the appearance of the wines made with the combination method was evaluated very close to the wines treated with mannoprotein.

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