EXAMINATION PHENOLIC MATURITY OF SYRAH GRAPE VARIETY

Doctoral (PhD) Theses

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1. INTRODUCTION

Nowadays wine consumers prefer well structured wines with deep color, fruit scents, soft tannins and pleasant mouthfeel. This kind of wines can be made from well-ripened with an optimal level of phenolic and technological (sugar) maturity, but not from overripened grapes. Nevertheless, the changing climate modifies the ripening process notably. In cool climate wine regions such as the Eger wine district in Hungary we can count on more frequent extreme weather events including uneven precipitation, heat waves, droughts, cool summer and warm winter. In dry and hot vintages the ripening process is faster, and the balance between phenolic and technological (sugar) maturity may not be maintained. This results in an increase in the sugar concentration, and in parallel, a rapid decrease in the titratable acidity resulting in unbalanced and too alcoholic wines. At the same time, the lack of optimal phenolic maturity results in wines with green and astringent tannins. On the other hand, in a rainy, cool vintage the ripening is slowed, and late ripening varieties (such as Cabernet Sauvignon, Cabernet Franc, Syrah) cannot reach optimal maturity. Several technological applications can be used in order to reduce these negative effects. Cluster thinning, girdling and early defoliation are reported to have a beneficial effect on phenolic maturity especially on anthocyanin and flavonoid synthesis. Beyond these techniques a new foliar spray (LalVigne® Mature) for enhancing phenolic maturity was developed recently, and it was examined for its effects. In addition, Syrah is a new cultivar to the Eger wine region, with only limited cultivation experience with it.

The aim of this study was:

1) to examine the impacts of different vintages (2011, 2012, 2013) and harvest dates on phenolic maturity of Syrah

2) to describe the effects of the application of this new foliar spray on grape phenolic maturity

3) to describe some aspects of the responses of a "new" variety (Syrah, *Vitis vinifera* L.) in a cool climate wine region (Eger, Hungary)

2. MATERIALS AND METHODS

Ten-year-old Syrah (clone ENTAV-INRA® 877) vines grafted onto Teleki 5C at a spacing of 2.4 m x 0.8 m with south-north row orientation were investigated at Nagy-Eged-hill. A trial site of 6 rows were selected for each treatment (3 control (unsprayed, C) and 3 treated (sprayed, LM) rows). Two puffer rows were left between the control and treated ones. Each row was divided into 3 blocks. One block contained 25-29 vines. At the same harvest time 3 blocks/treatment were harvested resulted in 3 replicates/treatment. The leaf spray, LalVigne® Mature is a formulation of 100% natural, inactivated wine yeast (*Saccharomyces cerevisiae*) derivatives (specifically designed to be used with the patent foliar application technology WO/2014/024039, Lallemand Inc., Canada). It is non-pathogenic, non-hazardous, food grade and non-GMO. Two applications of 1 kg/ha were done. The first one was at the beginning of veraison, the second one 12 days later. The powder was diluted in water without using an adjuvant. The whole canopy was sprayed with a motorized backpack sprayer.

Climatic data

Climatic data were monitored by an automatic weather station (Boreas Ltd. Érd, Hungary), approximately 300 m far from the trial site.

Berry sampling

Berry samples were collected 2, 3, 4, 6 and 8 weeks after veraison in 2011 and 2, 3, 4, 5, 7 weeks after veraison in 2012 and in 2013. Experimental wines were made at the last three harvest dates. Three sets of 20 kg grapes were carefully harvested for both treatments at each harvest date by hand, and transported immediately to the experimental winery.

Three one kg samples for each treatment were collected at random from several clusters before vinification. The berries were selected randomly from the upper, middle, and lower parts of the bunches. All the berry samples were prepared and analyzed within 2 hours after the harvest.

For the texture analysis, 50 berries were randomly removed from the clusters with pedicels and visually examined before texture analysis. One berry represents one repetition by this measurement. Damaged berries were rejected.

150 berries were separately selected for phenolic measurement (Glories method) and these berries were subdivided into two equal groups for the pH 1 and pH 3.4

solutions. The measurement was done in triplicate. 25 berries were used for each repetition.

Three additional sets of 100 grape samples were selected for weight determination and grape composition analysis.

Grape and wine analysis

The analytical methods recommended by the OIV (2014) were used to determine titratable acidity and the pH of the grapes as well as ethanol content, titratable acidity and pH of the wines. The sugar content was measured by Rebelein's method. The color intensity ($A_{420}+A_{520}$) and hue (A_{420}/A_{520}) of the wines were determined using the method described by Glories (1984). Phenolic components were measured by spectrophotometer (UVmini-1240 CE UV-VIS, Shimadzu, Japan). The bisulfite bleaching method was used to determine the anthocyanin content of grape extracts and wines (Ribéreau-Gayon & Stonestreet 1965). Total phenolics of the wines were analyzed by the Folin-Ciocalteu method (Singleton & Rossi 1965) and the results expressed as gallic acid equivalents (GAE mg/L). The quantity of leucoanthocyanidins (flavan-3,4-diols or procyanidins) was determined as described by Flanzy et al., (1969) while the total catechins (flavan-3-ols) were measured using the vanillin assay according to Amerine & Ough (1980). The gelatin and HCl indices (Ribéreau-Gayon et al., 2006) were also calculated. All the measurements were preformed in triplicate.

Assesment of grape phenolic maturity

The phenolic potential of grapes was calculated according to the method described by Saint-Cricq et al. (1998). This involved grinding the grapes with a blender and macerating for 4 hours with buffer solutions at two pH values (1.0 and 3.4). The original method proposed a pH 3.2 buffer, but this was adjusted to 3.4, as it is more relevant to the grapes from this region. The indices of phenolic maturity were calculated according to Glories & Augustin (1993): potential anthocyanins (A1), extractable anthocyanins (A3.4), cell maturity index (EA%) and seed maturity index (SM%). All the measurements were done in triplicate.

The following equations were used:

$$EA\ (\%) = [(A1 - A3.4) / A1] \times 100$$
$$SM\ (\%) = [(A280 - ((A3.4 / 1000) \times 40)) / A280] \times 100$$

Measurements of berry physical properties

A TA.XTplus Texture Analyzer (Stable Micro System, Surrey, UK) with HDP/90 platform and 30 kg load cell was used to follow grape physical properties and the Exponent 6.1.4.0 software was used for data evaluation. The main parameters were: skin break force (F_{sk}, N), skin break energy (W_{sk}, mJ), Young's modulus of berry skin (E_{sk}, N/mm), Berry skin thickness (Sp_{sk}), berry hardness (BH, N), seed break force (F_s, N), seed break energy (W_s, mJ) and Young's modulus of the seed (E_s, N/mm).

Qualitative and quantitative determination of resveratrol components in wines by HPLC

The analysis of resveratrol compounds was carried out according to Kállay & Török (1997). The eluent for the isocratic HPLC analysis consisted of a 5 : 5 : 90 mixture of acetonitrile : methanol : water. Operating conditions and chromatograph settings are as follows: a HP Series 1050 HPLC-apparatus with LiChrospher® 100, CN 5 µm column (Merck, Germany) was used during the measurements. The detector was a HP Series 1050. The flow was set 2 mL/min at 30°C with detection at 306 nm. *Trans*-resveratrol (99%) standard was purchased from Sigma-Aldrich (Germany). *Trans*-piceid standard was received from the San Michele all'Adige Research and Innovation Centre. *Cis*-isomers are produced by UV irradiation of the trans-isomers.

Microvinification process

Three sets of 20 kg grapes were crushed, destemmed and sulfited (1 mL of 5% aqueous SO_2 solution for every 1 L of mashed grape) in the experimental winery at each harvest date. Macerations were conducted in 30 L plastic containers, and all grape repetitions were separately fermented. Three experimental wine replicates were made at each harvest time for each treatment respectively. After grape processing the containers were transported immediately to the cellar to ensure constant ambient temperature (13°C) from the beginning to the end of maceration. After 24 hours of cold maceration selected active dry yeasts (20 g of dry yeast / 100 kg of processed grapes) (Uvaferm VN, Lallemand Inc.) and yeast nutrients (30 g / 100 kg of processed grapes) (Uvavital, Lallemand Inc.) were added. The maceration lasted for 23 days. The cap was punched down twice a day throughout the skin contact period. The wines were also inoculated with 10 mg/L lactic acid bacteria (Uvaferm Alpha, Lallemand Inc.) at the end of alcoholic fermentation. After 23 days the wines were pressed at 1.5 bar in a 30 L membrane press. Free-run and press wines were mixed. After malolactic fermentation had occurred, the wines were racked, and transported to the laboratory for analysis. All the wines were stored at 13°C until the moment of the analysis for several days, and no sulfur was added prior to analysis.

Sensory analysis

Blind tests were carried out by comparing in pairs the wines obtained from the three different harvest dates.

Statistical analysis

Statistical analysis was conducted by IBM SPSS 20 (IBM Corp., Armonk, NY, USA) software. Values were compared by multivariate ANOVA test with three factors (the effects of vintage: 2012, 2013, treatment: C (control), LM (LalVigne® Mature) and harvest dates) followed by between-subjects effect test. Homogeneity of variances was checked by Levene's test. In case of significant effect of harvest dates, Tukey's or Games-Howell post hoc test was used for mean separation, according to whether the homogeneity of variances were held or not.

3. RESULTS

Climatic characteristics

The weather of 2012 can be considered as dry (total rainfall was 439.2 mm compared to the 50-year average of 589.6 mm) and warm (average year temperature was 12.5°C compared to the 50-year average of 10.7°C). On the other hand, 2013 can be regarded as a moderate vintage (total rainfall: 663 mm, average year temperature: 12.2°C), although the weather was somewhat cooler with more rain during the flowering and ripening stage, than in 2012.

Berry weight, grape juice sugar concentration, acidity, pH, Glories indices

The grapes reached a greater level of technological maturity in 2012 (maximum sugar concentration: 237,7 g/l) compared to 2013 (maximum sugar concentration: 231,0 g/l). Indeed, the berry sugar concentration in 2012 exceeded 2013 by 15-25%. There were also notable differences in the case of titratable acidity with the values in 2013 being significant higher. The lowest concentration was 8.6 g/L. The foliar spray treatment had a significant effect on titratable acidity and pH of the grapes with the treated berries containing less acid. The weight loss of the berries during ripening is due to the dehydration. There was some rain between the second and the third harvest dates in 2012, however, which resulted in heavier berries. The Glories indices provide a good prediction on phenolic compounds in the resulting wines. In general, the lower the EA% and SM% values, the riper the berry. There was a positive effect of the leaf spray treatment on both total (A1) and potential (A3.4) anthocyanins, favoring their accumulation in both years and at nearly all harvest dates. A1 and A3.4 values indicate a good anthocyanin concentration especially in 2012. Interestingly, the EA% values showed an increase in some cases during ripening, implying that the extractability of the anthocyanins decreased. None of the factors affected the seed maturity index (SM%).

Grape texture properties

The impact of the leaf spray caused a significant increase in skin thickness (Sp_{sk}). The values were above 0.2 mm in the case of treated grapes at all harvest dates and in both years. There was no correlation between skin thickness (Sp_{sk}) and skin break force (F_{sk}) values. Changes in skin break energy (W_{sk}) showed a very similar pattern to F_{sk} related to the treatments and the harvest time. The berries became softer (BH) during the ripening. The significant increase observable in 2012 is due to the rainfall during the second and third harvest periods. The seed texture parameters remained unchanged despite the treatment between the harvest dates. However, the vintage had a very strong effect on these parameters.

Wine composition

The wines had a wide range of alcohol concentration (between 11.28 %v/v and 15.55 %v/v). The foliar spray did not influence this parameter, however. We found significant differences between the titratable acidity and pH in the first phase of the ripening, but the differences were no longer significant by the second and third harvest dates.

The total polyphenol values were independent of the foliar spray treatment. In 2012 we measured significantly higher (above 2,000 mg/L) values than in 2013 (concentration between 1,025 and 1,304 mg/L). The leucoanthocyanin and anthocyanin concentrations were found to be significantly higher in the treated wines in three instances: in 2012 at the second and the third harvest dates, and in 2013 at the second harvest date (although only for anthocyanins). The weather conditions in 2012 favored anthocyanin synthesis up to 796 mg/L. By contrast, in 2013, the unfavorable vintage resulted in significantly lower anthocyanin concentration. The color intensity ($A_{420}+A_{520}$) correlated well with the increasing concentration of anthocyanins. The values of color hue (A_{420}/A_{520}) represent bluish tone, but this is typical for young red wines. The impact of the foliar spray and harvest date on catechin levels is unclear. The gelatin index increased significantly in 2012 between the first and the third harvest dates in the foliar spray treated grapes. In 2013 the differences between harvest dates were smaller, and the values were also much lower than in 2012 and less than the optimal value due to the unfavorable weather conditions. During tastings the wines were characterized by green, unripe tannins. HCl indices show a marked variation from 4.34 to 12.99. The foliar spray treatment increased this parameter, but the difference was significant only at the second harvest date in 2012, and at the third harvest date in 2013. The majority of resveratrol was found in the wines as the isomeric forms of piceid (resveratrol glycoside). In 2012 and 2013, *cis*- and *trans*-resveratrol were not detected in the control wines at the first harvest date. *Trans*-resveratrol was also absent in 2013 in the treated wines in the second harvest date. Treated wines

contained this compound from the first harvest date. Under the effect of the foliar spray total resveratrol concentration increased especially in the first phase of ripening. The differences in total resveratrol concentration were not significant in three cases: at the second harvest dates in both years, and at the third harvest date in 2012.

4. NEW SCIENTIFIC RESULTS

Syrah needs a very good vintage an exceptional terroir under cool climate conditions in order to produce outstanding wines. This variety is highly sensitive to the weather at the ripening stage. The cool autumn in 2013 resulted in lower katechin, total polyphenol and anthocyanin concentration along with lower gelatin and HCl indices in comparison with 2012. In conclusion Syrah reached significantly lower phenolic maturity in 2013.

Evaluation of the changes in texture parameters of Syrah berries under cool climate conditions. The berry skin thickness and the seed texture parameters remained unchanged after veraison during the different harvest dates. The whole berry and the berry skin became softer as the ripening gone forward.

Enhancing phenolic maturity was mainly occurred due to the increase anthocyanin synthesis. The anthocyanin concentration was also higher at all harvest dates in the berries as well as in the wines treated with LalVigne® Mature. The extractability indices were also higher in some cases.

The impact of the leaf spray caused a significant increase in skin thickness (Sp_{sk}) at all harvest dates.

The foliar spray caused a significant difference between control and treated wines in the case of titratable acidity and resveratrol content in the early phase of ripening (first harvest date) in both vintages. The titratable acidity was lower and the resveratrol content was higher in treated wines.

All the tasters were able to differentiate between the control and treated wines, however the differences weren't significant statistically. In all cases, the tasters preferred wines made from treated grapes.

5. CONCLUSION

We examined the impacts of yeast derivatives applications (LalVigne® Mature, Lallemand Inc.) on Syrah grape phenolic maturity as well as wine phenolic composition and concentration. This foliar spray was tested on Syrah vines in two vintages (2012, 2013) in a cool climate wine region (Eger, Hungary). Experimental wines were made at three separate harvest times in each vintage. Standard analytical parameters for grapes and wines as well as resveratrol were evaluated. Changes in anthocyanin extractability and texture characteristics of the grape berries were followed during ripening. Grapes from treated vines had thicker skins than controls at all sampling dates in both vintages. Our experiment showed that phenolic ripening can be enhanced using the foliar spray. Therefore more balanced wines with more complexity could be produced without the danger of overripening. The results show that the application of the foliar spray is useful in different vintages. Preliminary evidence was also obtained to suggest that LalVigne® Mature may also help in cooler and less optimal vintages by enhancing the ripening process leading to wines with greater oenological potential. The phenolic maturity (especially anthocyanin concentration and its extractability) of the foliar spray treated grapes was greatly improved. The observed changes (the treated berries had higher anthocyanin and resveratrol content along with thicker skins) could be explained with vine-pathogen interaction. Vine recognizes the yeasts in the foliar spray, which is activating some defense mechanisms. In this way secondary metabolism is enhanced in the berries.

6. PULICATIONS

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