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Influence of Thermal Treatment on Polyphenol Extraction of Wine cv. André

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Abstract

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The extraction of polyphenols and colour parameter development of the grape variety André processed by thermal maceration techniques were examited. Comparing four different thermal maceration processes we have found out that the cold soak combined with classical maceration had the most significant effect on the extraction of total polyphenols and anthocyanins. Concentration of total phenols was more than 27% higher than in a thermovinificated sample. These techniques have also offered wines with the highest colour intensity. There was no considerable influence of maceration on the main products of ethanol fermentation but an effect on the concentration of total acids in wine was observed. In all cases fermentation caused a decrease of total phenols in André wines.

Keywords: temperature; wine; grape; colour

There are many methods of red grape processing used to produce different styles of red wines, all with one aim: to achieve more intense colour, full and balanced taste, and also the highest antioxidant capacity. As for old red grape varieties, the process of extraction of polyphenols during different types of maceration has been studied in detail but new varieties are not described so well.

Anthocyanins are the most important substances affecting the colour of red and rosé wine. Sacchi *et al*. (2005) noted that anthocyanin extraction reaches the maximum in early stages of fermentation and this concentration decreases while extraction of tannin increases proportionally to the continuing contact of grape skins and seeds. Postfermentation factors (maturing, aging) cause a decrease of free anthocyanin concentration and an increase of polymeric pigment content. No differences were observed in the anthocyanin content of wines made with addition of different sulphur dioxide levels at crushing (0, 50, and 100 mg/l) (Watson *et al*. 1995; Sacchi *et al*. 2005).

Thermal treatment. There are two ways of thermal treatment – heating and cooling. Heating the grape mash to a defined temperature can be carried out for a short (few minutes) or for a long time (few hours). Heat damages the hypodermal cell walls and membranes of grape berry tissues releasing anthocyanins. Heat also prevents browning due to denaturation of polyphenol oxidase. During thermovinification the concentration of anthocyanins increases twice (Gao *et al*. 1997). The highest anthocyanin content can be determined at the end of thermal treatment, and then during fermentation it decreases continuously (Gao *et al*. 1997). If the temperature does not exceed 65°C, there is no cooking tone in wine flavour (Steidl 2002). Wines prepared by this technology are suitable for fast consumption (Ribéreau-Gayon *et al*. 2006).

During short-time thermal treatment the grape mash is heated to 70°C and after 10 or 20 min it is cooled back to 20°C. Pressed must is rich in aromatic compounds and anthocyanins, but poor in tannins. By the action of heating grape berry cells are disrupted

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and colour pigments are released. This method is often used for the processing of low quality grapes to eliminate future bacterial contamination (Steidl 2002). Heating young wine together with grape mash to 35–40°C causes an increase of tannin content in mixture and resulting wine becomes more full-bodied. This type of technology cannot be used for the production of full wines from less ripe grapes.

Cold soak. Cold soak is a method when the grape mash is cooled to a low temperature and kept for several days. It could be done by cooling the whole fermentation tank or by using dry ice. Cold soak has been reported to have a low effect on the phenolic composition of resulting wines compared to freezing the mash before fermentation. Grape mash freezing causes berry cells to burst, cell walls to break and anthocyanins to be released (Sacchi *et al*. 2005). The use of solid carbon dioxide has an additional advantage: after freezing the grapes it sublimes on the surface of berries protecting them against the subsequent action of oxygen. It is interesting that this method reportedly increases the concentration of tannins while the heat treatment of berries enhances only anthocyanins. Freezing may also disrupt tannin-containing cells of the seeds, increasing total polyphenol extractability (Peinado *et al*. 2004; Sacchi *et al*. 2005). Ortega-Heras *et al*. (2012) reported that using dry ice to maintain the temperature is better for phenolics extraction.

New grape varieties have to be examined for the most suitable method for phenols extraction. This work deals with pre-fermentation conditions and their influence on phenolic parameters of wine. Various maceration techniques influence the sensory profile of wine differently.

MATERIAL AND METHODS

In this experiment was used the *Vitis vinifera* L. cultivar André (vintage 2013, collected October 26, 2013, concentration of reducing sugars 230 g/l, Malokarpatský winegrowing region, Modra winegrowing area, Suchý vrch vineyard, latitude 48°16'23.3004"W, longitude 17°21'51.7746"E). The André cultivar is a relatively new grape variety (approved by the law in 1980) which was created by crossbreeding of Blaufrankisch × Saint Laurent (seedling A16/76). In an experiment healthy grapes of cv. André were destemmed and berries were divided into the same parts with the same weight (400 g). Description of processes used in the experiment is presented in Table 1. Grapes used for the preparation of all experimental samples were crushed, treated with 30 mg/l $K_2S_2O_5$ (Sigma-Aldrich, Darmstadt, Germany) (antimicrobial and antioxidant agent) and macerated for 4 days. After this time all samples were pressed and 70 mg/l $K_2S_2O_5$ and 240 mg/l $(NH_4)_2HPO_4$ (both Sigma-Aldrich, Darmstadt, Germany) as a nitrogen source were added to all liquid samples (Baroň 2011). All samples were inoculated with 0.3 g/l active dry yeasts *Saccharomyces cerevisiae* var. *bayanus* FERMIVIN (Biowin, Łódź, Poland) and let to ferment in 500 ml flasks closed with fermentation stopper in cellar conditions (21°C). Sample 2 was macerated at 4°C for 4 days before classical maceration at 21°C. After 23 days of processing (pre-maceration, maceration, fermentation) the profile of polyphenols was determined in all samples of wines.

Determination of polyphenols. Concentrations of total phenols were determined by the alkali Folin-Ciocalteu method (Singleton *et al*. 1999) using the Folin-Ciocalteu reagent with gallic acid (both Sigma-Aldrich, Darmstadt, Germany) as a standard.

For detailed view on phenolic compounds, samples were analysed by Agillent 1100 Series HPLC (Agillent, Santa Clara, USA) with UV detector. Single polyphenols in samples (20 µl injection) were separated on a LiChrospher 100 RP-18 column (5 µm) (Merck Millipore, Darmstadt, Germany) with constant flow 0.5 ml/min. The mobile phase consisted of A (acetic acid : water = 1 : 99), B (acetic acid : water = 6 : 94),

Table 1. Description of used methods of grape processing

No.	Name	Description
1	control	classical maceration for 4 day at 21°C
2	cold soak	pre-maceration for 4 days at 4°C and than 4 days of classical maceration at 21°C
3	20% thermovinification	20% of mash thermally treated for 1 h at 65°C and added back to the rest of mash; then followed by classical 4 days maceration
4	20% cryomaceration	20% of mash cryomacerated for 4 days at –18°C, 80% of mash was macerated at 21°C
5	thermovinification	mash heated for 1 min at 45°C

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and C (acetic acid : water : acetonitrile = 5 : 65 : 30) and was changed according to the time schedule: 0–15 min 100% A → 100% B; 15–30 min 100% B; 30–50 min 100% B → 90% B and 10%C; 50–60 min 90% B and 10% C → 80% B and 20% C; 60–80 min 80% B and 20% C → 70% B and 30% C; 80–125 min 100% C; 125–130 min 100% C → 100% A. Single polyphenols were detected at defined detection wavelengths: gallic acid (97.5%) 272 nm, caffeic acid (≥ 98%) 320 nm, vanillic acid (≥ 97%) 272 nm, catechin (≥ 96%) 280 nm, polydatin (≥ 95%) (all from Sigma Aldrich) 320 nm.

Basic chemical analysis. Basic technological parameters of wine were determined by standard methods according to OIV (OIV-MA-AS-311-01A, 2009). Anthocyanins were determined by a modified method according to Somers (Somers & Evans 1977).

Statistical analysis. All results are described as mean values of triplicate measured samples. Standard deviation was calculated using MS Excel (Microsoft Office 2007; Microsoft, Redmond, USA).

RESULTS AND DISCUSSION

Comparing basic technological parameters (Table 2) of wine produced by various maceration techniques we can see that the final ethanol concentration and the concentration of residual reducing sugars are very similar. Differences were observed in particular organic acids (depending on the state of malolactic fermentation) and in the concentration of dry extract, which was the highest in sample 2.

During alcoholic fermentation colourless phenolics increase, reaching maximum values at pressing, and remain stable during malolactic fermentation and subsequent storage. Anthocyanin concentration increases during the early stages of alcoholic fermentation, reaching maximum values 2–3 days (3–6% v/v of ethanol) after the start of fermentation, and decreases during the storage of wine (Auw *et al*. 1996).

On the other hand, cold maceration ('cold soak') may be useful to increase anthocyanin to tannin ratio in lightly coloured vine varieties by increasing the time of extraction from the berry skins without the simultaneous increase in extraction from the seeds (Kovac *et al*. 1992).

By cold soak total anthocyanin concentration increased during pre-maceration from an average concentration of 64–102 mg/l (Balík *et al*. 2013). After 4 days of maceration total anthocyanins increased to 365–592 mg/l. During fermentation, the concentration of total anthocyanins increased, but finally, after fermentation total anthocyanin concentration decreased in all samples (270–403 mg/l) compared to the end of maceration. The highest concentration of total anthocyanins was determined in sample 3. Girard *et al*. (1997) observed a more than twofold increase of total anthocyanins during 60°C thermovinification. During the first days of fermentation we measured approximately 50% higher concentration of total anthocyanins. On the other hand, Gao *et al*. (1997) determined 3 times higher total anthocyanins (compared to the control) in the first days of fermentation.

The colour form of anthocyanins is only one part of total anthocyanins. At the end of fermentation, the highest value was recorded in cold soak. Similar results were reported by Gil-Muñoz *et al* (2008). Progress of total anthocyanins and their colour form

Table 2. Basic analytical parameters of André wines after fermentation

	Sample No.				
	1	2	3	4	5
Ethanol (% v/v)	10.7 ± 0.1	10.4 ± 0.1	10.7 ± 0.1	10.4 ± 0.1	10.4 ± 0.1
Glucose + fructose (g/l)	1.33 ± 0.21	1.47 ± 0.21	1.23 ± 0.24	1.09 ± 0.20	1.40 ± 0.21
pH (–)	3.28 ± 0.01	3.24 ± 0.01	3.06 ± 0.01	3.17 ± 0.01	3.24 ± 0.01
Total acids (g/l)	6.87 ± 0.07	7.91 ± 0.05	8.81 ± 0.07	7.55 ± 0.06	7.80 ± 0.09
Volatile acids (g/l)	0.28 ± 0.03	0.13 ± 0.03	0.24 ± 0.03	0.36 ± 0.03	0.17 ± 0.03
Malic acid (g/l)	0.34 ± 0.02	1.66 ± 0.05	1.81 ± 0.06	0.19 ± 0.02	1.59 ± 0.04
Tartaric acid (g/l)	3.42 ± 0.02	3.58 ± 0.03	3.70 ± 0.04	3.39 ± 0.03	2.98 ± 0.02
Lactic acid (g/l)	2.62 ± 0.06	1.09 ± 0.03	1.12 ± 0.04	2.55 ± 0.07	1.48 ± 0.05
Dry extract (g/l)	26.9 ± 0.1	30.9 ± 0.2	28.8 ± 0.1	27.6 ± 0.1	28.1 ± 0.1

Concentrations are expressed as mean ± standard deviation

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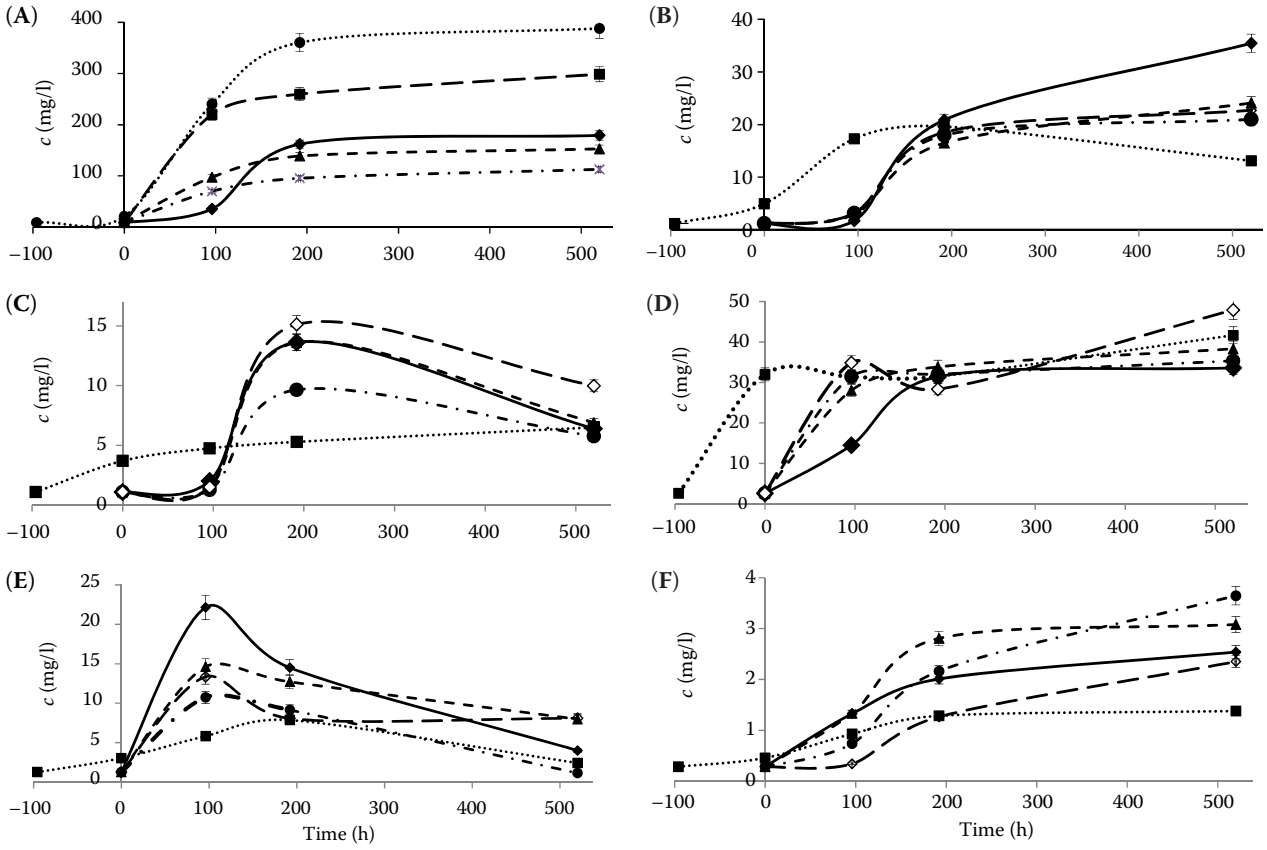

Figure 1. Progress of (**A**) gallic acid, (**B**) caffeic acid, (**C**)vanillic acid, (**D**) polydatin, (**E**) resveratrol, and (**F**) catechin extraction

Sample No.: ♦ – 1; ● – 2; ▲ – 3; × – 4; ■ – 5

have the same trend. The highest detected value of 131 mg/l (cold soak) decreased during fermentation to final 89 mg/l. The lowest value after 4 days of maceration (67 mg/l) was determined in sample 1, followed by the sample which was partially treated with cryomaceration. The trend of final concentrations of colour anthocyanins copies the values recorded after 4 days of maceration (Table 3).

Total phenolic content increased during cold soak only moderately (from initial 411.4 mg/l to 490.0 mg/l). Afterwards, during 4 days of maceration the concentration of total phenols increased 2–3 times. The highest concentration of total phenols was reached in sample 5 after 4 days of fermentation (1454.2 mg/l) and then during fermentation the concentration of total phenols decreased. These

Table 3. Anthocyanin concentration in André wine samples

Sample No.	Total anthocyanins (mg/l)				Color anthocyanins (mg/l)			
	4 days after			after fermentation	4 days after			after fermentation
	premaceration	maceration	fermentation		premaceration	maceration	fermentation	
1	–	365 ± 37	374 ± 32	327 ± 30	–	67 ± 6	69 ± 7	51 ± 6
2	102 ± 11	592 ± 37	511 ± 41	357 ± 30	21 ± 2	131 ± 12	93 ± 9	89 ± 8
3	–	439 ± 36	475 ± 35	403 ± 27	–	84 ± 9	90 ± 9	76 ± 9
4	–	387 ± 35	443 ± 32	334 ± 31	–	75 ± 8	75 ± 8	52 ± 7
5	–	436 ± 36	544 ± 36	270 ± 25	–	83 ± 8	90 ± 9	77 ± 8

Concentrations are expressed as mean ± standard deviation

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Table 4. Concentration (mg/l) of total phenols

Sample No.	4 days after			After fermentation
	premaceration	maceration	fermentation	
1	–	1122.1 ± 23.3	1094.3 ± 22.7	708.5 ± 22.3
2	490.0 ± 22.4	1555.0 ± 23.9	1454.2 ± 24.1	974.7 ± 23.7
3	–	1086.4 ± 21.3	1022.9 ± 20.9	644.4 ± 22.1
4	–	968.6 ± 19.0	944.3 ± 19.7	587.0 ± 23.3
5	–	1222.1 ± 21.3	1094.3 ± 22.0	762.1 ± 22.6

Concentrations are expressed as mean ± standard deviation

results correlate with Budić-Leto *et al*. (2005). In their work, samples reached the maximum total phenolic content after the first 5 days of fermentation. Total phenolics decreased in all samples to 587.0–974.7 mg/l. On the other hand, Heredia *et al*. (2010) found differences in the concentration of total phenols between cold soak (1288.3 mg/l) and freezing grapes (2841.6 mg/l).

Gallic acid showed an increase during maceration and first days of fermentation. The greatest increase (388 mg/l) was detected in cold soak. The second highest increase of gallic acid (298 mg/l) was determined in a sample treated with thermovinification at 45°C. Control sample showed a slower beginning (Figure 1a), but during fermentation the concentration of gallic acid increased to 179 mg/l. According to Borazan and Bozan (2013) this increase of gallic acid can be caused by hydrolysis of gallotannins at defined pH.

The concentration of caffeic acid rose for the whole time (Figure 1B), except for sample 2, where its concentration decreased after 4 days of fermentation. Maximum concentration was detected in control sample (35 mg/l) at the end of fermentation.

Vanillic acid showed a rapid increase between the fourth and eighth day of processing (Figure 1C), except for sample 2. Finally, it seems that thermal maceration techniques do not significantly influence the final concentration of vanillic acid.

During maceration polydatin concentration increased the most (Figure 1D). Then during fermentation, the concentration of polydatin decreased. It can be caused by β-glucosidase (E.C. 3.2.1.21), which can degrade polydatin to resveratrol. Comparing polydatin and resveratrol concentrations, we can see that the concentration of resveratrol rises parallelly with polydatin decrease. The highest concentration of polydatin was detected in control sample, but during fermentation it fell to one half. After fermentation, the concentration of polydatin diminished to a concentration similar to that at the beginning. The highest concentration of resveratrol was extracted in sample 4 (Figure 1E).

Catechin was extracted continuously for 4 days with almost the same rate (Figure 1f), except for the control sample, where extraction took a twice longer time. Finally, all the concentrations were almost the same, except for sample 5.

CONCLUSION

When comparing the basic parameters of wine, all winemaking techniques influence the wine minimally. Total phenolic content was highest in a sample treated with cold soak. In all cases, fermentation caused the decrease of total phenols in wines. The lowest phenolic content was recorded in a sample with 20% cryomaceration.

The lowest total anthocyanin content was measured after fermentation in control sample and sample with 20% of cryomacerated fraction. In most samples total anthocyanin concentration reached the highest values after 4 days of fermentation. During fermentation their concentration decreased. The highest diminution was observed in a cold soaked sample where the concentration of total anthocyanins decreased from 592 mg/l to 357 mg/l.

Taking all together, cold soak positively influences the tested colour and basic analytical parameters of André wine in the most important way. The lowest increase of total phenols, anthocyanins, and colour intensity was recorded in control sample treated only with the addition of potassium disulphite.

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