Food Bioprocess Technol (2016) 9:421–429
DOI 10.1007/s11947-015-1637-2

ORIGINAL PAPER

Influence of Technological Processes on Biologically Active Compounds of Produced Grapes Juices

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Received: 27 May 2015 / Accepted: 26 October 2015 / Published online: 5 November 2015

Abstract Grape juice quality can be substantially influenced by different processing methods and their parameters. This study deals with the influence of thermomaceration temperature and its holding time on important juice quality parameters such as the content of *trans*-resveratrol, *trans*-piceid, *trans*-ε-viniferin, and 2,4,6-trihydroxyphenantrene-2-*O*-glucoside. All the aforementioned compounds together with antioxidative capacity, total polyphenols, and antimutagenic activity were measured. This study used the following grapes: Blaufränkisch, Blaufränkisch bio, Saint Laurent, Grüner Veltliner, Grüner Veltliner bio, and Müller-Thurgau. For all tested grape varieties, the most important processing parameters were temperature and thermomaceration holding time. There were no significant differences in the content of the studied compounds. Three preservation technologies, freezing, bottle pasteurization, and high-pressure treatment, were also studied regarding the content of abovementioned biologically active compounds. Results of this study can be immediately applied to grape juice production.

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Keywords Grapevine juices · Thermomaceration · Biologically active compounds · Antioxidative capacity · Total polyphenols · Antimutagenic activity

Introduction

Living healthy lifestyles is a worldwide trend today, and consumers are increasingly attentive to health-beneficial bioactive compounds contained in foods. Grape juice is a commercially interesting commodity, mainly in the form of pure musts and/or various non-alcoholic beverages containing high proportions of grapes. Producing soft drinks based on grape musts can help to solve the problem of overproduction of low-quality table wines (Aurand 2015). At the same time, more and more consumers are looking for organic products, and it can be expected that this trend will increasingly strengthen also in the area of manufacturing drinks from grapes.

Methods for commercial processing of grape juice have undergone continuous change. In the 1990s, the term hot-press juice processing appeared. That process is carried out by heating grapes, adding pectolytic enzymes, and then holding the crushed berries at 60 to 63 °C for 30 to 60 min under slow-moving agitation (Morris 1998). In one study, the musts from fresh or frozen grapes of Cabernet Franc, Cabernet Sauvignon, Chambourcin (an interspecific hybrid), and Cynthiana were heated to 71 °C for approximately 20 min. The total amount of phenolics was thereby increased in a range from 43 to 619 % as compared to the juice from unheated must (Threlfall et al. 2006). Liu et al. (2015) recently described the affect must thermomaceration on polyphenol content in wines.

A great number of epidemiological studies have demonstrated that regularly and adequately consuming a wide spectrum of antioxidants decreases the risk of cardiovascular,

tumor, and neurodegenerative diseases (Watson et al. 2014). This prevention is based on scavenging of reactive oxygen species (ROS), defense against oxidative damages, and protection from potential mutations of cellular DNA (Halliwell 1990; Hertog et al. 1993; Stoner and Mukhtar 1995; Madhavi et al. 1996; Scalbert and Williamson 2000; Park et al. 2003). Estimating the level of total antioxidant capacity (or activity) is becoming one of the most important factors in food quality evaluation within the field of modern nutrition (Fogliano et al. 1999; Pellegrini et al. 2003; Dávalos et al. 2005; Stratil et al. 2007).

The fruit of *Vitis vinifera* L. and products made of grapes constitute a good source of numerous natural phenolic antioxidants, such as resveratrol, piceid, non-flavonoid, and flavonoid phenols (including anthocyanin pigments) (Rice-Evans et al. 1996; Cui et al. 2002; Dong 2003; Singletary et al. 2003; King et al. 2006). Pace et al. (1996) reconfirmed the fact that the alcohol alone contained in wine has a greater protective effect against atherosclerosis and coronary heart disease than the content of the various phenolic compounds contained in white and red wines. On the other hand, increased levels of polyphenols and, above all, of biologically active *trans*-resveratrol in non-alcoholic grape musts very probably lead to reduced risk for atherosclerosis. Another clinical study demonstrated that increased consumption of grape musts heightened the antioxidant activity of blood plasma and that probably was associated with reduced damage of leucocytes (Park et al. 2003). Keewil et al. (2000) emphasized that grape juice (but not orange and/or grapefruit juice) showed positive effects on platelet aggregation. This means that this inhibitive effect of polyphenols contained in grape musts (if regularly consumed) may decrease the risk of coronary thrombosis and myocardial infarction.

Both the concentration and composition of bioactive substances present in grape musts are influenced by a great number of factors, including grape variety, health of the grapes, growing conditions, and—above all—the processing technology. The aforementioned phenolic antioxidants are present mainly in the skin, while their concentration in the pulp of the berries is very low. Musts produced in a conventional way (i.e., by means of immediate pressing and subsequent preservation) have a low content of antioxidative and health-beneficial phenolic compounds, either because of their minimum extraction from skins or due to their low stability caused by inattentive processing of grapes and manufacturing of juices (Fuleki and Ricardo-Da-Silva 2003; Gonzales-Manzano et al. 2004; Villaño et al. 2006; Downey et al. 2006; Dani et al. 2007; Moreno et al. 2008). There is very limited information available in the literature regarding how grape musts' bioactive substances change during processing, which is why this paper studies methods to influence juice quality parameters such as *trans*-resveratrol, *trans*-piceid, *trans*-ε-viniferin, and 2,4,6-trihydroxyphenantrene-2-*O*-glucoside content, together with antioxidative capacity, total polyphenols, and antimutagenic activity with regard to thermomaceration temperature and thermomaceration holding time. Such research results can help producers optimize the technological process and substantially increase the quality of processed grape juice.

Materials and Methods

Plant Materials

This study involved two white grapes varieties—Müller-Thurgau (MT) and Grüner Veltliner (GV)—and two red varieties—Blaufränkisch (BF) and Saint Laurent (SL). Grapes originating from conventional viticulture were produced in vineyards from the Mendel University, Brno, and training farm at Lednice, Czech Republic. BF grapes originated from organic production (labeled as "bio") from the Gotberg winery in Popice, Czech Republic. Organic Grüner Veltliner grapes were produced at the Binder Winery in Rakvice, Czech Republic. Grapes were grown under standard conditions of wine agriculture from three harvests years: 2010, 2011, and 2012.

Must Preparation

Grape batches weighing 100 kg were destalked, crushed, and, after supplementation with L-ascorbic acid (100 mg/kg), macerated as follows: maceration of grape mash (30 kg) without heating at ambient temperature 20–25 °C for 60 min (designated A), thermomaceration of grape mash (30 kg) at 80 °C for 20 min (designated D20), and thermomaceration of grape mash (30 kg) at 80 °C for 40 min (designated D40). Parameters of tested thermomaceration were predicted by preliminary experiments. Holding times longer than 40 min were found to have no effect on analyzed substances. The most effective temperature was found 80 °C.

Musts were pressed using a laboratory pneumatic press (up to 0.2 MPa) and racked after 2 h of standing at a temperature of 5 °C. Grape juices (20 L each) from the three thermomacerations (A, D20, and D40) were subjected to various preservation technologies (heat pasteurization, freezing, and high-pressure treatment). The musts were pasteurized (20 min at 85 °C) in 0.5-L dark glass bottles with crown caps and stored at 5 °C, freeze-dried at −18 °C in plastic bags, or treated at 500 MPa for 10 min in plastic bags. Musts were analyzed within a week of processing. Analysis was always carried out on using three parallel samples.

Sensory Evaluation

Sensory assessments of the pasteurized grape juices were produced using a graphical hundred-point scale, and an ordinal method was used by a panel of ten selected sensory assessors (under the ISO standard). The non-parametric sensory data were evaluated using the Friedman rank test.

Antioxidant Determination

Concentrations of *trans*-resveratrol and its derivatives were determined in samples of grape musts using high-performance liquid chromatography and a Phenomenex Luna C18 (2) column, with a mixture of acetonitrile, water, and *ortho*-phosphoric acid as the mobile phase and a diode array (DAD) and fluorescence (FLD) detection in accordance with Tříska et al. (2012). Quantification of *trans*-resveratrol and *trans*-piceid was performed using authentic standards (Sigma-Aldrich) while *trans*-ε-viniferin was quantified using a *trans*-resveratrol standard. 2,4,6-Trihydroxyphenantrene-2-*O*-glucoside was quantified using 9-phenanthrol as a standard (Sigma-Aldrich). Total polyphenols were estimated using the Folin–Ciocalteu solution, and the results were calculated as gallic acid (Singleton and Rossi 1965). The antioxidant capacity was determined by ferric reducing antioxidant power (FRAP) assay using 2,4,6-tripyridyl-s-triazine at 620 nm and the DPPH method using 2,2-diphenyl-1-picrylhydrazyl radical at 515 nm. Antioxidant capacity was calculated from the calibration curve using Trolox (Balík et al. 2008). Antimutagenic activity was measured according to the literature using the Ames test with IQ mutagen (2-amino-3-methyl-3H-imidazo-(4,5-f)quinoline) (10 ng/100 μL) and *Salmonella typhimurium* TA 98 with metabolic activator S9 (Totušek et al. 2011).

Antimutagenic Activity

Antimutagenic activity was evaluated as inhibition rate I (%) using the following formula:

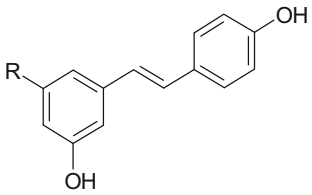
$$I\ (\%) = 100 - ([Rt\ (\text{tested sample} + \text{mutagen})/Rt\ (\text{mutagen})] \times 100),$$

where Rt = number of revertant colonies. According to the following evaluation scale of inhibition:

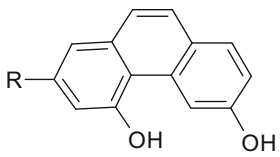
0 – 20 = negative
20 – 40 = weakly positive
40 – 60 = positive
60 – 90 = strongly positive
90 and more = probably toxic

Statistical Analysis

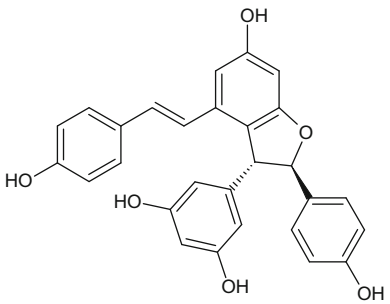
Statistical analysis of the data was performed using UNISTAT 5.1 and Statistic 8. ANOVA was used to indicate differences between experimental results.



a R...OH *trans*-resveratrol
b R...O-glucopyranoside *trans*-piceid



c R...O-glucopyranoside 2,4,6-trihydroxyphenanthrene-2-*O*-glucoside (THPG)



d *trans*-ε-viniferin

Fig. 1 Chemical structures of stilbene and phenanthrene derivatives

Results and Discussion

Trans-resveratrol and its derivatives or transformation products were found mainly in the skins of individual berries (Fig. 1). Because their water solubility is low, their extraction requires either an adequately long maceration period. The application of enzymes or the effect of ethanol produced in the course of fermentation can be considered to increase extraction efficiency.

Heating the crushed grapes causes not only disintegration of those structures in cell membranes that contain *trans*-resveratrol derivatives, but also acceleration of the process of extracting all phenolic components (including anthocyanin pigments) into the must. Levels of both *trans*-resveratrol and

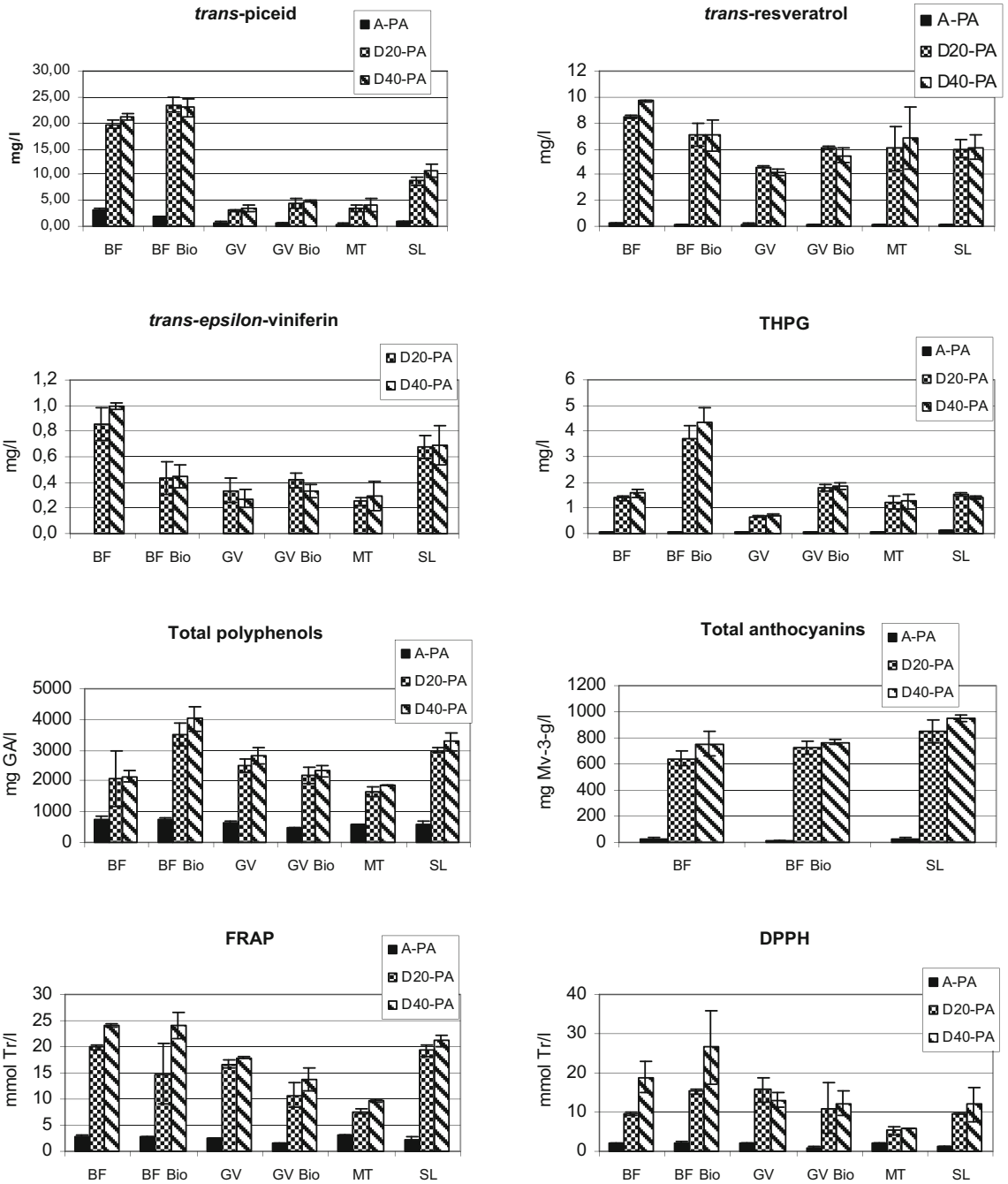


Fig. 2 Amount of *trans*-piceid, *trans*-resveratrol, *trans*-ε-viniferin, 2,4,6-trihydroxyphenantrene-2-*O*-glucoside (THPG), total polyphenols, total anthocyanins, and antioxidant capacity determined using the ferric reducing antioxidant power (FRAP) assay and by the DPPH method using 2,2-diphenyl-1-picrylhydrazyl radical in relation to the maceration process (*A* ambient temperature, *D20* thermo-maceration, *D40* thermomaceration) and grape variety (Blaufränkisch (BF), Saint Laurent (SL), Müller-Thurgau (MT), and Grüner Veltliner (GV). All grapes were from the 2010 harvest

piceid in musts without application of thermomaceration ranged from 0.10 to 0.23 mg/L and from 0.46 to 3.12 mg/L, respectively (see Fig. 2). The average amounts of *trans*-resveratrol and *trans*-piceid in grape musts after thermomaceration increased by an average of 50 and 9 times, respectively. The best results were obtained after 20 and 40 min of thermomaceration at 80 °C with crushed BF grapes. The contents of *trans*-resveratrol and *trans*-piceid were 7.0–

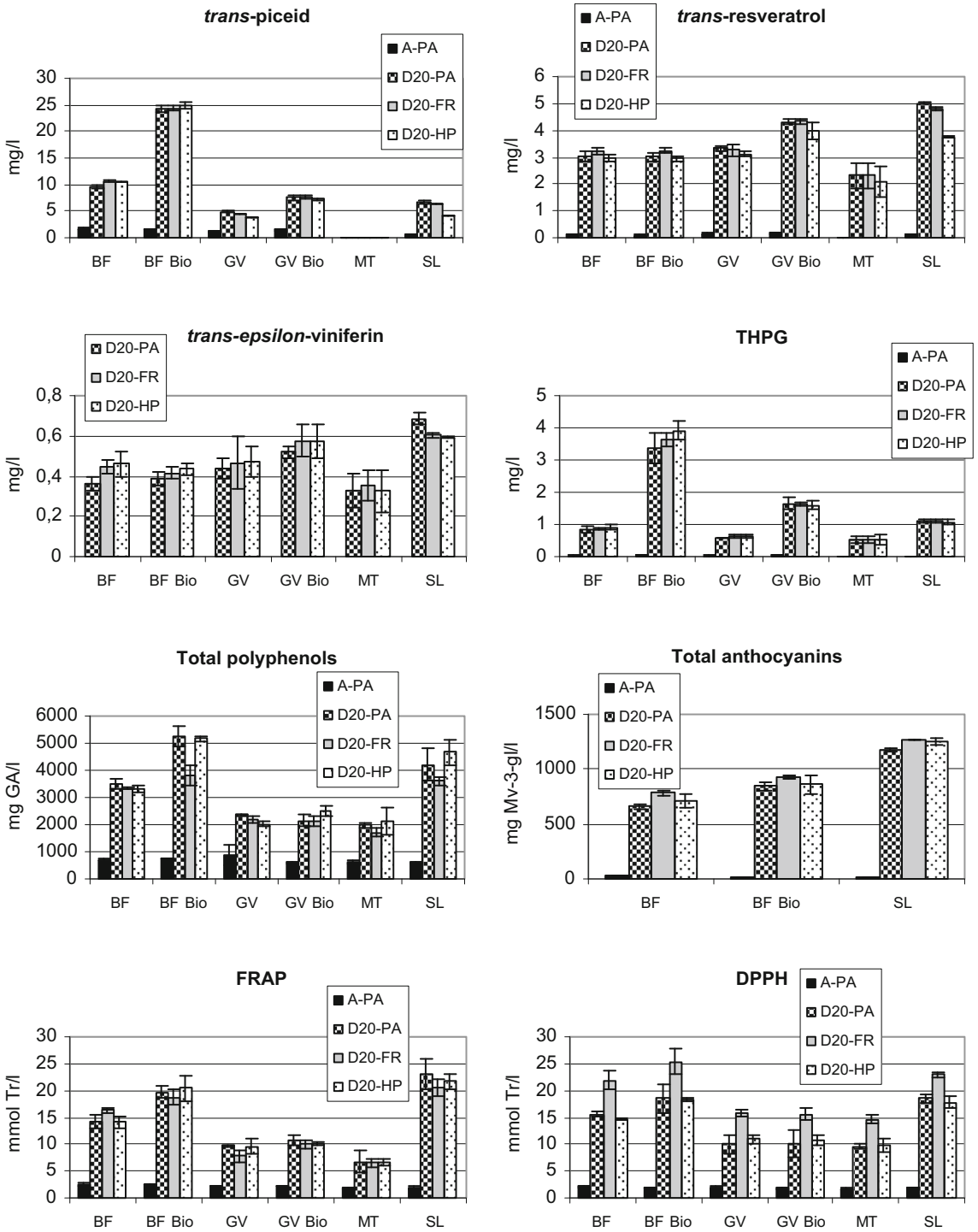


Fig. 3 Amount of *trans*-piceid, *trans*-resveratrol, *trans*-ε-viniferin, 2,4,6-trihydroxyphenantrene-2-*O*-glucoside (THPG), total polyphenols, total anthocyanins, and antioxidant capacity determined using the ferric reducing antioxidant power (FRAP) assay and by the DPPH method using 2,2-diphenyl-1-picrylhydrazyl radical in relation to preservation technology (*PA* heat pasteurization, *FR* freezing, *HP* high-pressure treatment) and grape variety (Blaufränkisch (BF), Saint Laurent (SL), Müller-Thurgau (MT), and Grüner Veltliner (GV). All grapes were from the 2011 harvest

9.7 and 19.7–23.5 mg/L, respectively. The standard deviation values (Figs. 2, 3, and 4) indicate that the positive effects of thermomaceration comparable to those for ambient temperature maceration (A) on the contents of *trans*-resveratrol, *trans*-piceid, *trans*-ε-viniferin, and 2,4,6-trihydroxyphenantrene-2-*O*-glucoside (THPG) in grape juices were statistically significant ($p < 0.05$) for all varieties. Meanwhile, the effect of 40 min of maceration (instead of 20 min) exhibited no significant effect ($p > 0.05$) on the levels of these compounds in comparison with 20 min of maceration (Fig. 2). Similar

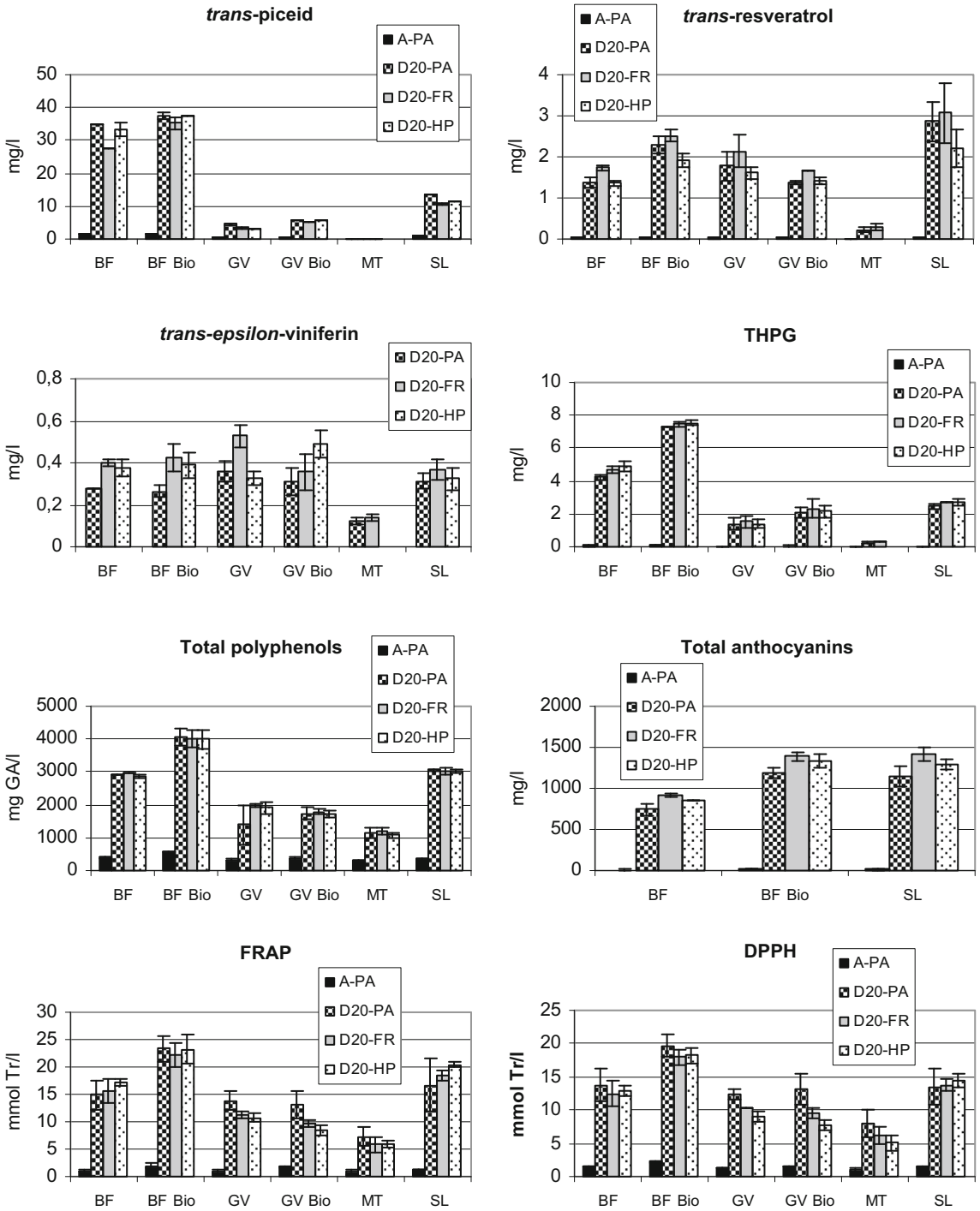


Fig. 4 Amount of *trans*-piceid, *trans*-resveratrol, *trans*-ε-viniferin, 2,4,6-trihydroxyphenantrene-2-*O*-glucoside (THPG), total polyphenols, total anthocyanins, and antioxidant capacity determined using the ferric reducing antioxidant power (FRAP) assay and by the DPPH method using 2,2-diphenyl-1-picrylhydrazyl radical in relation to preservation technology (*PA* heat pasteurization, *FR* freezing, *HP* high-pressure treatment) and grape variety (Blaufränkisch (BF), Saint Laurent (SL), Müller-Thurgau (MT), and Grüner Veltliner (GV). All grapes were from the 2012 harvest

correlations were also found with regard to the values of total polyphenols, total anthocyanins, and the antioxidant capacity of grape juice musts.

Thermomaceration of grapes showed a fundamental effect on the dynamics of biologically active substance transfer from skins into the must, and this was positively reflected in increasing antioxidant capacity values of the grape musts thus produced. In samples of juices without thermomaceration, the antioxidant activity ranged from 1.46 to 3.06 mmol of Trolox/L (FRAP) or from 0.96 to 2.11 mmol of Trolox/L (DPPH). Thermomaceration caused a statistically significant ($p < 0.05$) increase in levels of substances having antioxidant properties. This was reflected in a gain by as much as ten times in values of antioxidative activities (i.e., from 19.9 to 24.0 mmol of Trolox/L). It was again confirmed that heating crushed grapes to 80 °C for a period of 20 min was sufficient and that it was not necessary to extend the period of maceration to 40 min, since doing so did not significantly increase the antioxidant capacity of juices.

As in other studies, a significant correlation was seen between the content of total polyphenols and values of antioxidant activity (FRAP and DPPH) of musts ($r = 0.8881$ and $r = 0.8695$, respectively) (Dani et al. 2007). After heating, the most dynamic increase in the level of polyphenols was recorded in musts made from BF bio grapes, and in this case, the value was increased by approximately 5.4 times (from 739 to 4023 mg/L). While phenolic components very often constitute a source of bitter and constringent taste tones, this expectation was not substantiated by sensory tests of the produced musts. Both the taste and overall sensory quality of the thermomacerated musts were evaluated positively by nearly all respondents. The correlation coefficient values indicate that the qualitative composition of polyphenols and of other non-phenolic antioxidants present might be the main cause of the measured differences in antioxidant activity values for the grape musts under study. This was similarly reported by Dávalos et al. (2005).

In contradiction to Dani et al. (2007), who had compared grape musts made of organically and/or conventionally produced grapes, and with the sole exception of THPG, our study revealed that no significant differences ($p > 0.05$) between values of antioxidant capacity and *trans*-resveratrol, *trans*-piceid, *trans*-ε-viniferin, and/or total polyphenols content were found between grapes produced in organic versus conventional growing systems. A more pronounced influence on the content of antioxidant bioactive components was observed in the case of individual varieties and, above all, of grape processing technologies. This phenomenon had been observed also by Fuleki and Ricardo-Da-Silva (2003). The content of THPG was statistically higher ($p < 0.05$) in all juices from bioproduction with the exception of Grüner Veltliner and Grüner Veltliner bio from 2012. It seems that the combination of abiotic and biotic effects in organic production could significantly increase the concentration of THPG (Tříska et al. 2012). Although these findings must be proven again, it seems that the concentration of THPG in juices from bioproduction, or the ratio of THPG concentration in juices from bioproduction to the THPG concentration in juices from conventional production, might be used as a marker for the authenticity of juices from organic bioproduction.

We also studied the content of the aforementioned compounds in the prepared juices after three different methods of processing: freezing, bottle pasteurization, and high-pressure treatment. There was no general tendency that would

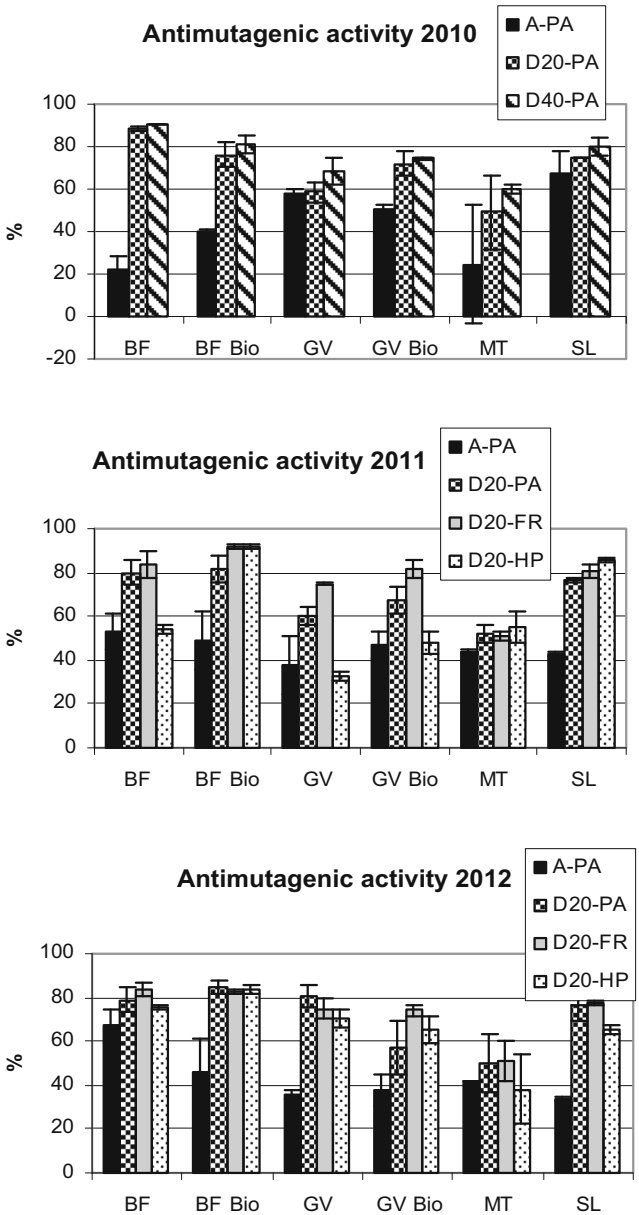


Fig. 5 Comparison of antimutagenic activity of different juices (2010 harvest) prepared at ambient temperature (A) and at 80 °C for 20 min (D20) or at 80 °C for 40 min (D40), using different preservation technologies (*PA* heat pasteurization, *FR* freezing, *HP* high-pressure treatment). The D20 process used grapes from the 2011 and 2012 harvests

suggest a preference for one or another particular processing method. All juices and all technologies provided (with a few exceptions) had almost the same concentrations of studied compounds and almost the same values of antioxidative capacity, total polyphenols, and antimutagenic activity. Some exceptions were found, e.g., for total polyphenols and variety BF bio (Fig. 3), for *trans*-ε-viniferin and variety Grüner Veltliner (Fig. 4), and in antimutagenic activity for BF, Grüner Veltliner, and Grüner Veltliner bio varieties in 2011. However, pasteurization is an appropriate technology from a total technological cost point of view.

Most of the juices had strongly positive antimutagenic activity with the exception of the MT variety, which demonstrated only a positive effect (Fig. 5). In general, the increase in antimutagenic activity was not as distinct as was the increasing content of those biologically active compounds studied.

Conclusions

Thermomaceration of grapes resulted in a significant increase in levels of *trans*-resveratrol, *trans*-piceid, *trans*-ε-viniferin, and THPG as well as total polyphenols, total anthocyanins, and the antioxidant capacity of produced grapevine juices. The best results and substantial increase of all studied quality parameters were achieved with thermomaceration for 20 min at 80 °C. Although there were some differences in the content of the studied compounds or in the studied parameters of the final prepared juices, with respect to heat pasteurization, freezing, and high-pressure treatment as preservation technologies, the most suitable preservation method from an economics viewpoint is probably heat pasteurization.

Acknowledgments The authors remember Dr. Jiří Totušek, who died in December 2013, for his collaboration. This research was supported by the Ministry of Agriculture of the Czech Republic (Grants No. QI91B094 and QJ1210258) and by the Ministry of Education, Youth and Sports of CR within the National Sustainability Program I (NPU I) (grant number LO1415).

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