Dynamics of changes in total anthocyanins during the fermentative maceration of grapes

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ABSTRACT: This paper deals with the results of studies on changes in the content of total anthocyanins depending on their fermentative maceration from grapes of traditional Moravian cultivars (Blauer Portugieser, Saint Laurent and Blaufrankisch). Colouring matters were macerated in the course of alcoholic fermentation either in a closed rotary tank or in an open tank with a periodically plunging pulp cap. The losses resulting from adsorption of anthocyanins on yeast biomass were quantified. Concentration losses of anthocyanins were also observed in the course of fermentation of musts made of teinturier grape cultivars Alibernet and Neronet, when the skins of berries were separated. The process of fermentative maceration of anthocyanins from grape skins consisted of three dynamically different stages. In the stage with the exponential increase in the content of anthocyanins no significant differences were observed between the closed rotary tank technique and the open tank technique with periodic plunging of pulp cap. It was demonstrated that the losses of anthocyanins took place during the whole process of fermentation. The correlation between the anthocyanin losses and the production of yeast biomass was statistically highly significant.

Keywords: vinification; anthocyanin extraction; anthocyanin losses; yeast biomass

The destruction of cell integrity in berries containing anthocyanins is the starting point of active physical and biochemical maceration processes that take place until equilibrium is established between anthocyanins concentrations in the solid and liquid phase. The pace of the process leading to the balanced concentrations of anthocyanins is determined by the degree of disintegration of plant material on the one hand and by its mechanical movement on the other. When making red wines, this process is accelerated by stirring, supplementation of sulphur dioxide, enzymes, alcohol production during fermentation, changing pressure of carbon dioxide and by heating of grape mush; all these steps can take place in open vats as well as in fully automated closed fermentors (Mandžukov 1989; Hamatschek, Pototschnigg 1990; Zimman et al. 2002). Vinification of red wines is based on alcoholic fermentation and maceration, i.e. dissolving of components present in grape must and in solid particles of grapes. There are three basic technologies of red wine making, viz. vinification based on (*i*) crushing of grapes and a simultaneous fermentation and maceration; (*ii*) heating of grapes when the process of maceration is separated from fermentation; (*iii*) maceration with carbon dioxide combined with anaerobic fermentation which takes place in intact berries (Troost 1980; Vivas et al. 1992). Sims and Bates (1994) recorded the maximum rate of anthocyanins extraction between days 4 and 6 of fermentation of crushed grapes. A longer maceration resulted in an increase in the content of polyphenols, which was positively manifested as late as during the storage and maturation of red wine (Gómez-Plaza et al. 2001). After seven days of fermentative maceration, Romero-Cascales et al. (2005) observed only minimum changes in the content of anthocyanins and they therefore discussed the effects of adsorption and degradation of yeast cell walls. Mandžukov (1989) mentioned that after the stage of the maximum concentration of anthocyanins in the liquid phase of wine their concentration decreased either due to their degradation or their adsorption on particles that did not contain these pigments. The cultivar Cabernet Sauvignon contained 1,250–1,550 mg of anthocyanins per 1 kg of grapes but only 25–40% of these colouring matters

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occurred in wine. Cuinier (1988) emphasized that the yeast strain is also very important because it can considerably influence the overall profile of phenolic substances in produced wine as well as the amount of adsorbed pigments. The adsorption capacity of yeast biomass is significantly influenced by the structure of present anthocyanins and by other physico-chemical parameters of red wines. The degree of adsorption increases with decreasing pH, and multipolar anthocyanins, especially those with several free hydroxyl groups, are adsorbed preferentially. It is also important whether the anthocyanin molecule is acylated by remnants of either acetic or cumaric acids (Vasserot et al. 1997; Medina et al. 2005).

The aim of this study was to analyze the dynamics of changes in the content of total anthocyanins during the fermentative maceration of grapes and to quantify their losses due to their adsorption on yeast biomass.

MATERIAL AND METHODS

The process of anthocyanin maceration from the skin of berries was studied in the course of alcoholic fermentation taking place in a closed rotary tank and/or in an open tank with periodic plunging of pulp cap. Grapes of three cultivars traditionally grown in Moravia (i.e. Blauer Portugieser, Saint Laurent and Blaufrankisch) were used. Must samples were taken in regular time intervals within a period of 12 days to analyze concentrations of extracted total anthocyanins and produced alcohol. Besides, losses of anthocyanins in the course of fermentation of teinturier grapes (cultivars Alibernet and Neronet) were studied. In this case, skins were separated from berries prior to the onset of fermentation.

The following parameters of separated yeast biomass were estimated: total weight, dry matter (at 105°C) and, after an extraction with acidified methanol, content of total anthocyanins using a spectrophotometric method (Heloisβ/Unicam) as developed by Fuleki and Francis (1968) and modified by Balík (1994). Concentration of total anthocyanins pigments in fermenting musts and young red wines were estimated in three replications using the spectrophotometric method (Heloisβ/Unicam) described by Somers and Evans (1977). Concentrations of alcohol in grape must samples taken during

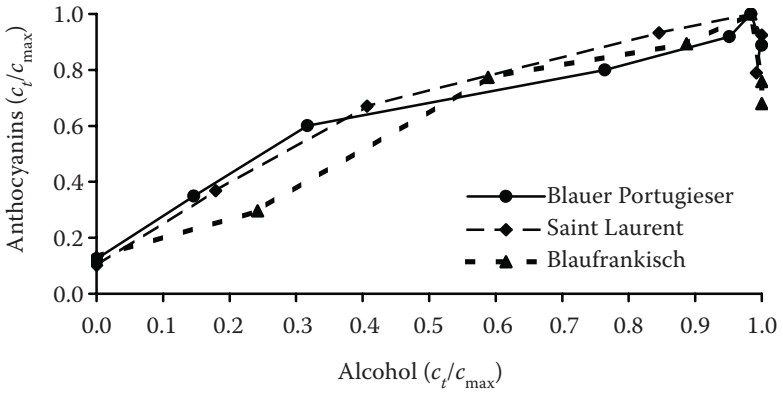

Fig. 1. Maceration of anthocyanins of three cultivars during grape fermentation in closed rotary tank depending on alcohol production

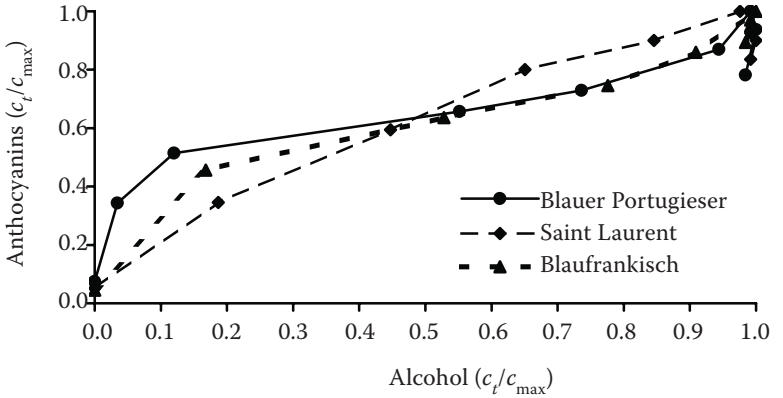

Fig. 2. Maceration of anthocyanins of three cultivars during grape fermentation in open tank with periodic dipping of pulp cap depending on alcohol production

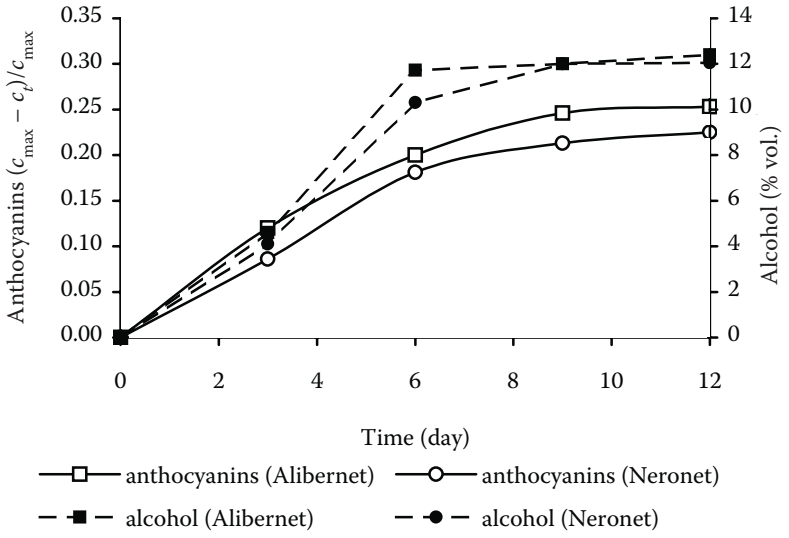

Fig. 3. Losses of anthocyanins and production of alcohol during fermentation of teinturiers without grape skins

fermentation were estimated using oxidometric titration (Zoecklein et al. 1990). Statistical methods of the Unistat software were used for the correlation analysis, cluster analysis and analysis of variance.

RESULTS AND DISCUSSION

Experiments were carried out in three different wineries that processed the same cultivars with different concentrations of colouring matters in harvested grapes. For the sake of comparison of maceration dynamics occurring in individual vinification methods all analytical concentrations of anthocyanins (c_t) were expressed as shares of maximum concentrations (c_{max}) recorded in individual samples within the terminal stages of fermentation. The course of alcohol production was documented in the same way (Figs. 1 and 2). At the beginning of anthocyanins estimation, their concentrations corresponded with levels estimated in grape musts after crushing and removal of grape stems. Estimated differences in concentration of pigments corresponded to the delicacy of methods used for the pre-fermentation of harvested grapes and to conditions of mush transportation into fermentors in individual wineries. Both maceration technologies enabled to reach at least 12.0% vol. of alcohol. In the closed rotary tanks, however, the fermentation process reached the level of maximum alcohol concentration as soon as on the day 6 while in the open tank with periodic plunging of pulp cap (cultivars Blauer Portugieser and Saint Laurent) this maximum was reached on day 8.

The increase in anthocyanin concentrations, recorded in given time intervals, was closely correlated with the production of alcohol, which was the most important factor of maceration of colouring matters in all studied cases. The maximum concentrations of anthocyanins were reached one day earlier than those of alcohol. The course of anthocyanin

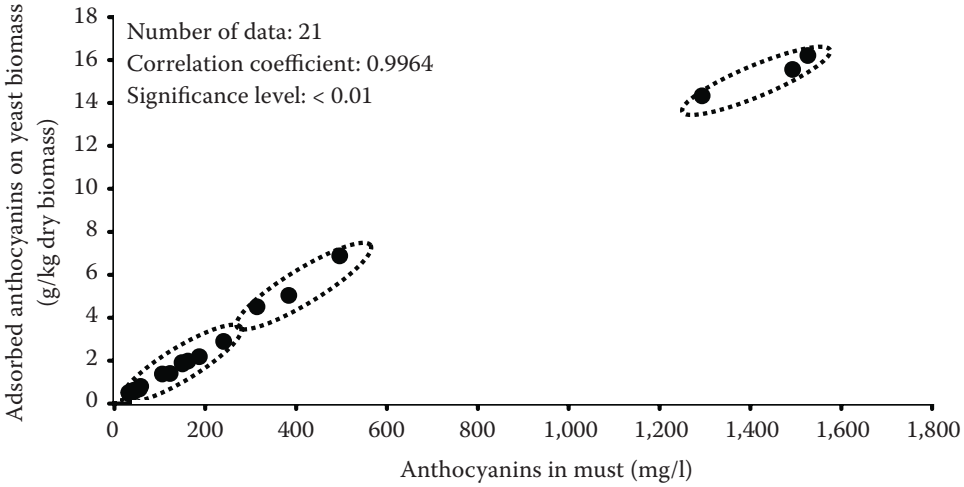

Fig. 4. Correlation and cluster analysis between total anthocyanins in musts and anthocyanins adsorbed on yeast biomass

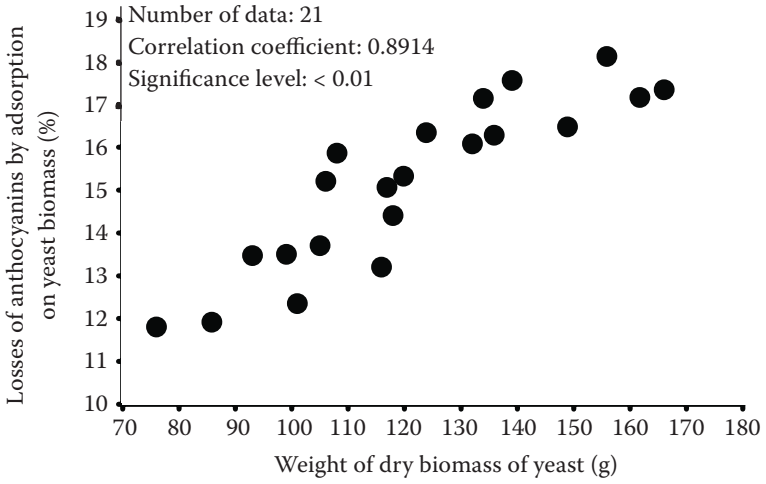

Fig. 5. Correlation analysis between weight of yeast biomass and loss of anthocyanins by adsorption on yeast biomass

extraction can be defined as a process consisting of three different stages: at the beginning (Stage 1), the highest rate of maceration was observed, even quicker than that of alcohol production; in Stage 2 the dynamics of anthocyanin concentration increase slowed down markedly as compared to the intensity of alcoholic fermentation. In the end of fermentation (Stage 3) (alcohol: c_t/c_{max} = 0.95–1.00), there was a striking decrease in the contents of anthocyanins by 11% to 32% (anthocyanins: c_t/c_{max} = 0.68–0.89) as compared to their maximum concentrations. This observation corresponded with findings of many authors who interpreted this decrease as losses of anthocyanins due to their destruction and adsorption on yeast biomass (Figs. 1 and 2).

To eliminate the effects of the interfering process of extraction of anthocyanins from skins the subsequent experiments were carried out using teinturier grapes of cultivars Alibernet and Neronet, which were fermented without skins. On the day 1 of fermentation, the content of anthocyanins in separated grape musts corresponded with their maximum concentrations ($c_o = c_{max}$). Within a period of twelve days, their losses $(c_{max} - c_t)/c_{max}$ were observed and the course of alcoholic fermentation was monitored simultaneously with the estimation of produced alcohol (Fig. 3). Losses of anthocyanins increased within the whole process of fermentation and the average loss of anthocyanins reached the limit of 19.1% on day 6. Fermentation losses of anthocyanins in young wine with the maximum of 12.0 % vol. of alcohol represented in average 24% of the initial concentration of colouring matters in musts of teinturier grapes. There was a highly significant correlation (0.9964) between the concentration of anthocyanins in musts (young red wines) and the amount of anthocyanins adsorbed on yeast biomass. Using the cluster analysis it was possible to divide experiments in three groups with different percentages of anthocyanins adsorbed on produced yeast biomass (Fig. 4). The extent of anthocyanin losses was greatly influenced by their initial concentrations in grapes of individual cultivars and by the amount of produced yeast sediments. There was a highly significant positive correlation between losses of anthocyanins and the total weight of sedimented yeast biomass (Fig. 5). Results of the analysis of total anthocyanins present in separated yeast sediments documented that their adsorption ranged, depending on the colour potential of raw material, from 0.43 to 16.21 g/kg dry yeast biomass. This factor, however, represented only 15% of total losses of anthocyanins recorded till the first racking of young wine. The remaining losses were caused by other biochemical and physico-chemical processes that took place during the process of fermentation and production of young wine.

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Dynamika změn veškerých antokyaninů v průběhu fermentační macerace hroznů

ABSTRAKT: V práci byly sledovány změny veškerých antokyaninů v závislosti na jejich fermentační maceraci z hroznů odrůd tradičně pěstovaných na Moravě (Modrý Portugal, Svatovavřinecké, Frankovka). Během alkoholické fermentace v uzavřeném rotačním tanku a v otevřeném kvasném zařízení s periodicky ponořovaným matolinovým kloboukem byly kvantifikovány ztráty barviv způsobené adsorpcí na kvasničnou biomasu. Koncentrační ztráty antokyaninů byly sledovány rovněž během kvašení červených moštů odrůd barvířek (Alibernet, Neronet), kdy byly před startem fermentace separovány slupky hroznů. Průběh fermentační macerace antokyaninů ze slupek hroznů byl popsán jako proces se třemi dynamicky odlišnými fázemi. V části exponenciálního zvyšování koncentrace barviv nebyl ze statistického hlediska zaznamenán významný rozdíl mezi postupy v rotačním tanku a v zařízení s otevřeným matolinovým kloboukem. Bylo prokázáno, že ke ztrátám antokyaninů dochází v celém průběhu kvasného procesu. Statisticky byla potvrzena vysoce významná korelace mezi koncentračními ztrátami antokyaninů a vyprodukovanou hmotou kvasničné biomasy.

Klíčová slova: vinifikace; extrakce antokyaninů; ztráty antokyaninů; kvasničná biomasa

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