Effect of bentonite clarification on concentration of anthocyanins and colour intensity of red and rosé wines

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ABSTRACT: The objective was to follow losses of the concentration of total anthocyanin pigments in red and rosé wines clarified with various doses of two commercial types of bentonite and to perform a sensory and analytical evaluation of differences in colour intensity of clarified and non-clarified wines. The loss of anthocyanins increased with the increasing doses of bentonite but statistically significant differences existed only between doses of 0.5 and 1.5 g/l. Not the type of bentonite but its dose showed an important effect on the extent of changes in colour intensity of wine. A sensory evaluation of colour intensity showed that in rosé and red wines doses of 0.5 g/l and 1.5 g/l, respectively, reduced significantly the intensity of wine colour as compared with non-clarified samples.

Keywords: wine; clarification; bentonite; total anthocyanins; colour intensity

The appearance of grape wine is an important sensory parameter and for potential consumers it is the first evaluated organoleptic property of this beverage. The genuine colouration of red and rosé wines can be fully perceived only in brightly clear red and rosé wines. This means that for many wine producers colour represents an important tool for evaluation of various vinification methods, technological parameters and qualitative innovations of production (DEL CARO et al. 1994; ROGGERO et al. 1988).

In red grapes of the species *Vitis vinifera* L., malvidin-3-monoglucoside is the predominating pigment. DRDÁK et al. (1991) estimated concentrations of anthocyanins in seven varieties of *Vitis vinifera* L. and in five interspecific samples of red grapes and obtained the results ranging from 5.40 to 15.55 g/kg of skins. Using HPLC, altogether 14 different anthocyanins were identified and their amounts ranged from 88.62 to 97.92% of all present anthocyanin pigments. In red grapes, both the content and the quality anthocyanins are strongly dependent on the variety. WÜRDIG and WOLLER (1989) mentioned that in the varieties Shiraz, Cabernet Sauvignon and Pinot Noir concentrations of anthocyanins were 2,200 mg/kg; 1,700 mg/kg and 825 mg/kg of grapes, resp.

The total level of evaluation of the colour potential of grapes is modified by a number of factors of the maceration process, e.g. enzymatic activity, fermenting activities of microorganisms, treatment with sulphur dioxide, duration of maceration process, etc. However, the final concentration of pigments in young red wines is closely associated not only with applied vinification procedures, methods and conditions but also with the original concentration of anthocyanins in grapes. This parameter is primarily dependent on the genetic predisposition of each variety to accumulate these pigments but, besides, it is also influenced by climatic and agrotechnical factors of each vintage (HAMATSCHEK, POTOTSCHNIGG 1990; EDER 1997).

It is known that each clarification reduces the content of both extractive and volatile substances. Until now, bentonite has represented the most reliable and also ecological clarifying preparation that assures protein stability especially in white wines. In addition to the capacity to induce precipitation of proteins, bentonite is capable to adsorb phenolic compounds and, thus, also anthocyanins (WÜRDIG, WOLLER 1989). Clarification of wines with sodium-calcium bentonites resulted in lower losses of pigments than that with calcium bentonites and the colour of treated red wines was more stable (GÖRTGES 1982; LESKE et al. 1995). With regard to frequent spontaneous stabilisation of proteins with tannins and to the adsorptive capacity of bentonite to bound phenols of plant origin (i.e. also anthocyanins) this clarifying agent is not recommended for routine clarification of red wines because it increases the risk of colour losses (TROOST 1988). In wine-making practice, however, it is possible to find red wines with a high concentration of thermo-unstable proteins and simultaneous presence of protective colloids that show a negative effect on the natural stabilisation of wine and complicate processes of wine treatment without application of bentonite.

The aim of experiments was to investigate concentration losses of total anthocyanins in red and rosé wines after their clarification with two commercial types of bentonites and to evaluate differences in colour intensity

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of clarified and non-clarified wines by means of both analytical methods and sensory evaluation.

MATERIAL AND METHODS

In our experiments altogether four samples of red (Samples 1, 3, 5, 7) and four samples of rosé wine (Samples 2, 4, 6, 8) were used. Samples of wine originated from wine district Mikulov (the varieties: 1 = Neronet; 2, 3 = Frankovka; 4 = St. Laurent; 5–8 = new selections – interspecific hybrids). Bentonites of trade marks NaCalit 2000 (Erbslöh Geisenheim) and Sabenil 550 (Neuber Praha) were applied into young, once drawn wines in the following doses: 0.0 g/l (control), 0.5 g/l, 1.0 g/l, 1.5 g/l. Bentonite suspension (5%) was prepared in such a way that the weighed amount of bentonite was mixed with distilled water and let swell for 24 hours. Individual volumes of bentonite suspension (0, 1, 2 or 3 ml) were then mixed with 50 ml of tested wine and thoroughly stirred and diluted with aliquot volumes of distilled water to reach identical dilutions of all clarified wines. Clarified samples of wine were thereafter filled up with the same wine to the 100 ml mark in a graduated cylinder and thoroughly stirred again. Cylinders were closed with aluminium foil and let stand for 24 hours at 5ºC; thereafter the liquid part of clarified wines was decanted and filtered through a glass frit S4.

The ranking was applied for sensory evaluation of colour intensity of clarified wines using 30ml glass test tubes. Data of the panel were evaluated in such a way that the ranks of individual evaluators were added together and statistical significance of these results was calculated using the sign test in Kramer's modification (NEUMANN et al. 1990).

The quantitative estimation of total anthocyanins and their losses in clarified wines was performed using the spectrophotometric method developed by FULEKI and FRANCIS (1968) and modified by PRÍBELA (1987). Colour intensity of clarified wines was measured directly by means of spectrophotometry as the sum of absorbencies at wavelengths 420, 520 and 620 nm (SOMERS, EVANS 1977). Significance of differences between mean values was tested using simple analysis of variance of the programme Unistat. Tukey's test was used for subsequent tests and the significance of differences between means was documented graphically by confidence intervals at the significance level $\alpha = 0.05$. Statistical significance of relationships between concentration of anthocyanins and colour intensity of clarified wines was evaluated on the basis of the number of variables and the value of correlation coefficient using the Unistat programme.

RESULTS AND DISCUSSION

Concentrations of anthocyanins in original, non-clarified wines (C-samples) ranged from 15 to 596 mg/l. This wide interval of anthocyanin concentrations was reflected in estimated values of colour intensity of experimental wines (0.27–8.92); this enabled to evaluate the possibility of bentonite clarification also from the aspect of anthocyanin concentrations in original wines (Tables 1 and 2). The highest losses of anthocyanins were recorded in samples with a low content of pigments (Samples 2, 4) and, surprisingly, also in Sample 7 with the initial concentration of anthocyanins equal to 102 mg/l. In the variant with the clarifying dose of 1.5 g/l of Nacalit or Sabenil, the losses of anthocyanins made as much as 18.9–30.0%. According to VALUIKO (1989) the losses of original colour of wines clarified with bentonite were as high as 40%.

In the sample with the highest concentration of anthocyanins (Sample 5), the losses of pigments made only 5.8% and 9.0% after the application of Nacalit and Sabenil (in the dose of 1.5 g/l), respectively (Figs. 1 and 2). A high variability of anthocyanin losses was caused partly by considerable heterogeneity of original concentrations of anthocyanins in clarified wines and partly by their different qualitative composition that could show a marked influence on their adsorption capacity (VASSEROT et al. 1997). After the application of the lowest test doses of Nacalit and Sabenil (0.5 g/l), absolute losses of anthocyanins ranged from 1–12 mg/l and from

Table 1. Concentrations of anthocyanins in red and rosé wines as dependent on the degree of clarification with bentonites Nacalit and Sabenil

Sample	C	Anthocyanins (mg/l)					
		Nacalit (g/l)			Sabenil (g/l)		
		0.5	1.0	1.5	0.5	1.0	1.5
1	90	85	85	82	86	84	77
2	15	14	14	12	14	13	12
3	154	142	138	131	144	136	122
4	38	31	29	29	34	30	27
5	596	590	579	561	586	577	542
6	50	50	46	45	49	47	44
7	102	91	85	75	94	88	71
8	45	43	42	37	42	40	35

C = control (no clarification sample)

Table 2. Colour intensity of red and rosé wines as dependent on the degree of clarification with bentonites Nacalit and Sabenil

Sample	C	Colour intensity					
		Nacalit (g/l)			Sabenil (g/l)		
		0.5	1.0	1.5	0.5	1.0	1.5
1	3.49	2.89	2.77	2.70	2.94	2.73	2.59
2	0.27	0.23	0.22	0.19	0.23	0.21	0.19
3	4.74	4.45	4.17	3.75	4.49	4.00	3.58
4	0.49	0.47	0.44	0.43	0.47	0.43	0.41
5	8.92	8.62	8.47	8.09	8.59	8.21	7.94
6	1.59	1.50	1.42	1.34	1.48	1.43	1.31
7	2.03	1.51	1.40	1.34	1.57	1.44	1.30
8	1.34	1.20	1.13	1.06	1.18	1.07	1.02

C = control (no clarification sample)

1–10 mg/l, resp., while for the highest doses of Nacalit and Sabenil (1.5 g/l) they ranged from 3–35 mg/l and 3–54 mg/l (Tables 1 and 2).

Another reason for the occurrence of fluctuating losses of anthocyanins during the process of clarification probably consisted in a different capacity of individual types

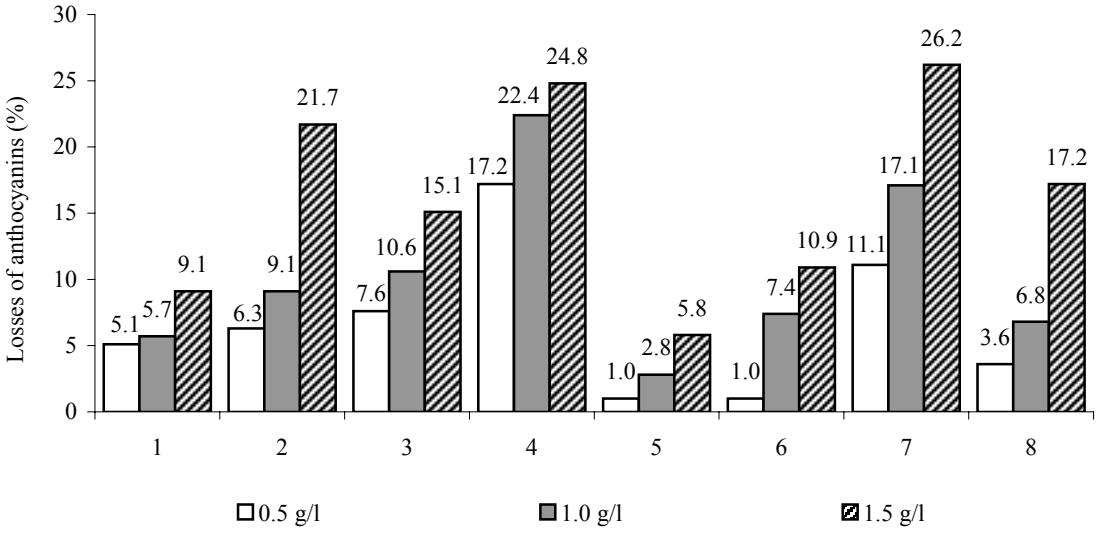

Fig. 1. Losses of anthocyanins in wines clarified with different doses of bentonite Nacalit

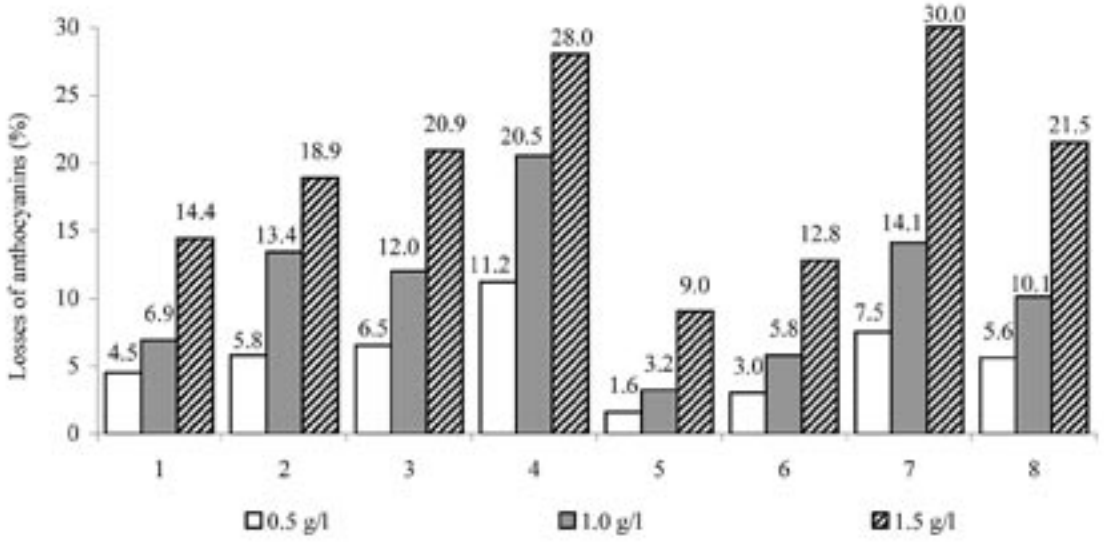

Fig. 2. Losses of anthocyanins in wines clarified width different doses of bentonite Sabenil

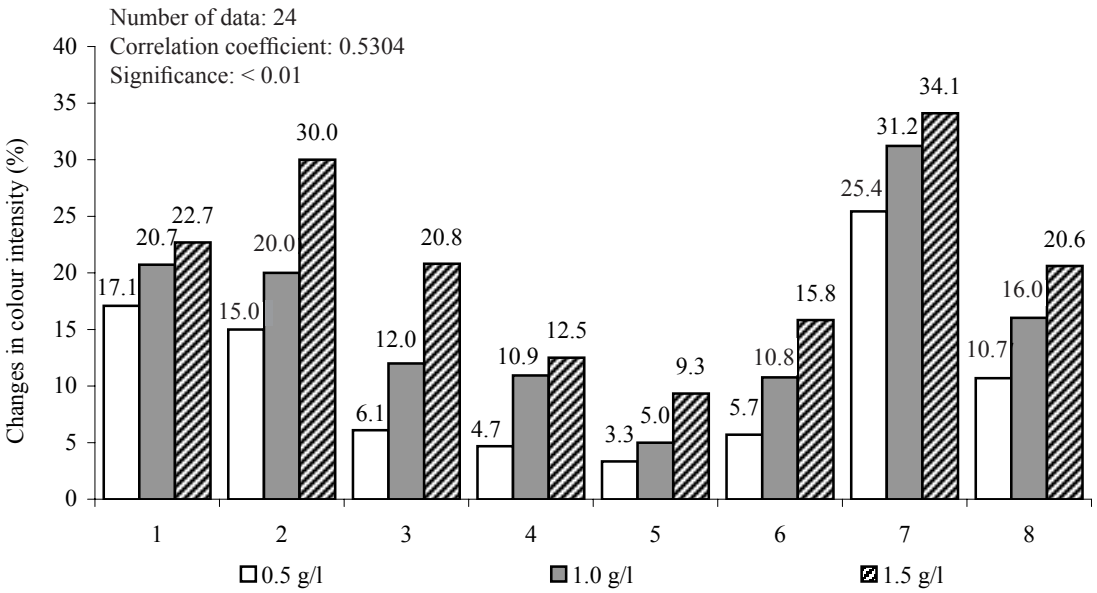

Fig. 3. Changes in colour intensity of wines clarified with different doses of bentonite Nacalit and analysis of correlations between losses of anthocyanins and changes in intensity of wine colour

of bentonite to bind anthocyanins, i.e. in the different size of the formed active adsorption surface (LESKE et al. 1995). However, the total area of this active adsorption surface could not result only from the properties of individual types of bentonite; undoubtedly, it could also be modified by the action of other wine components that were precipitated due to the clarification (GÖRTGES 1982). This could explain differences in the losses existing between samples with very similar initial concentrations of anthocyanins. For example in Sample 1, which was clarified with Nacalit, these losses were 5.1–9.1% while in Sample 7 (also with Nacalit) the losses were more than doubled (11.1–26.2%). The differences in wine composition were obviously caused by the occurrence of different physicochemical changes during the process of clarification and this was also the cause of disproportions in increasing losses observed in samples with increasing doses of bentonite (Figs. 1 and 2).

A great variance in measured losses of anthocyanins was also manifested in the extent of changes in colour intensity of clarified wines as estimated by means of spectrophotometric measurements (3.3–35.8%). The existence of statistically highly significant correlations between losses of anthocyanins on the one hand and changes in analytical values of colour intensity on the other was demonstrated for both types of bentonite. Lower values of correlation coefficients (0.5304 and 0.6286) indicated that there were several other important factors that influenced the extent of changes in the colour of clarified wines, e.g. the quantity and quality of

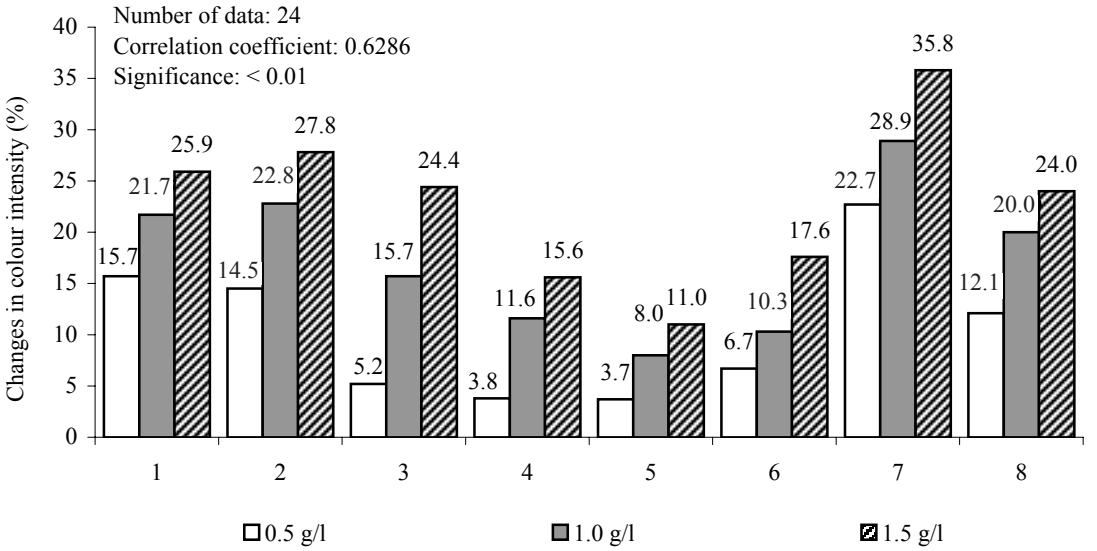

Fig. 4. Changes in colour intensity of wines clarified with different doses of bentonite Sabenil and analysis of correlations between losses of anthocyanins and changes in intensity of wine colour

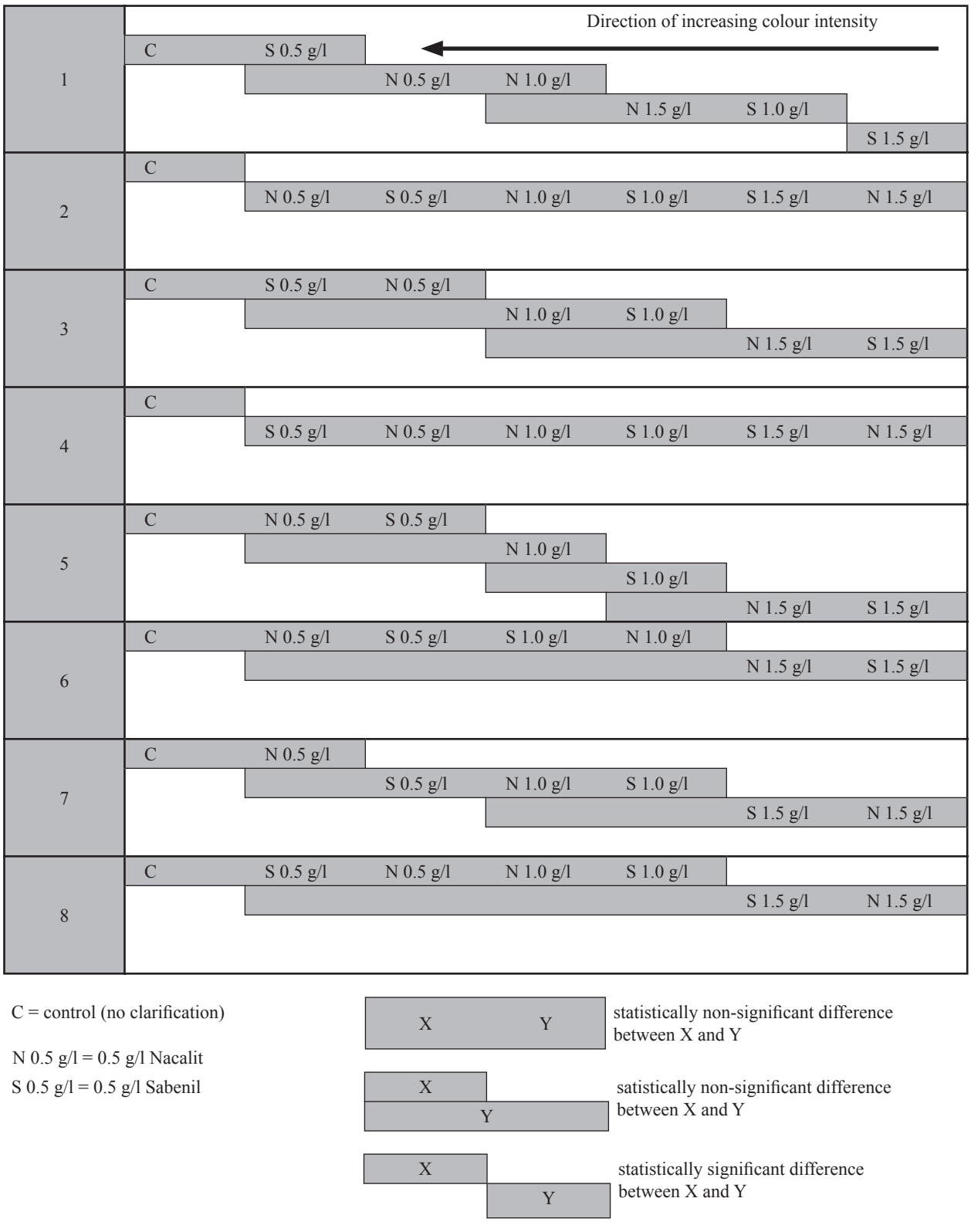

Fig. 5. Sensory differences in colour intensity of wines clarified with bentonites as estimated by the ranking (Kramer, $\alpha = 0.05$)

anthocyanins, pH of wine, concentration of sulphur dioxide and possibly also the presence of co-pigments and co-polymeric partners of anthocyanins (Tables 1 and 2, Figs. 3 and 4).

As far as the sensory evaluation of colour intensity of clarified wines was concerned, no statistically significant differences were found altogether in ten cases between control, i.e. non-clarified (C-samples) and clarified (0.5 g/l) samples of wine, regardless of the type of bentonite used. After the elimination of results obtained with Samples 2 and 4, which as non-clarified samples were more or less similar to the claret type of wine, it

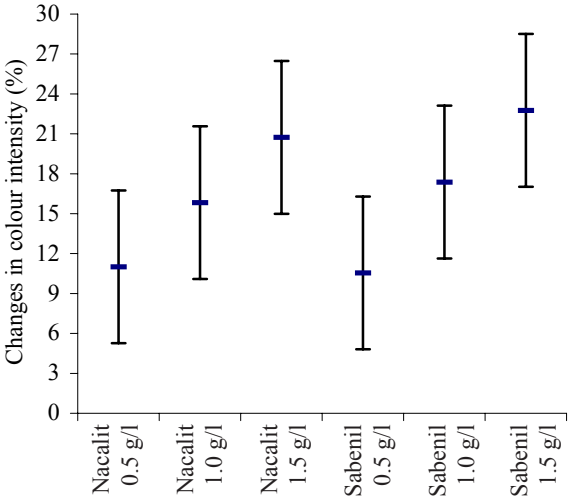

Fig. 6. Simple analysis of variance of changes in colour intensity as dependent on the type and dose of bentonite (Tukey, $\alpha = 0.05$)

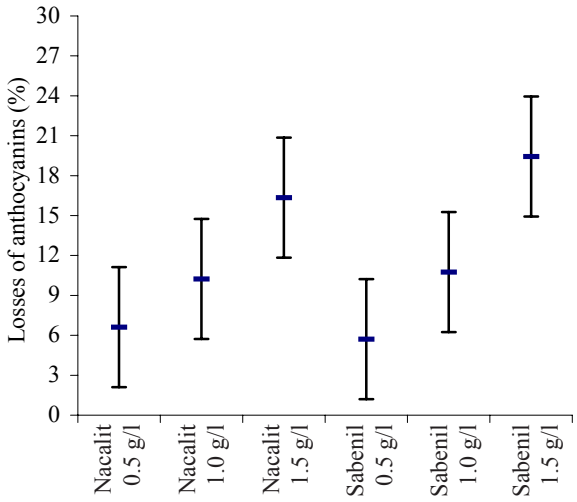

Fig. 7. Simple analysis of variance of concentration losses of anthocyanins as dependent on the type and dose of bentonite (Tukey, $\alpha = 0.05$)

was possible to conclude that the applied dose (0.5 g/l) of both Nacalit and Sabenil showed a negative effect on recorded differences in colour intensity only once (i.e. in Samples 1 and 7). On the other hand, sensory evaluations enabled to conclude that the doses of 1.5 g/l of Nacalit and/or Sabenil significantly reduced the intensity of colouration of all clarified wine samples compared to non-clarified ones (Fig. 5).

The analysis of variance of one-dimensional classification of changes in colour intensity as dependent on the type and dose of bentonite showed that the type of clarifying agent was not important and that a statistically significant difference existed only between wines clarified with 0.5 g/l and 1.5 g/l of Sabenil. This demonstrated the fact that the dose of bentonite is only one of the factors influencing the final colouration of clarified wines. Colour intensity is also influenced by a number of other factors, above all by physical composition and by physicochemical properties of clarified wines and non-clarified wines. When limiting the evaluation of bentonite effect on the process of clarification of red and rosé wines only to the examination of changes in anthocyanin concentrations it was experimentally demonstrated that the losses of anthocyanins markedly increased in dependence on increasing doses of bentonite. However, statistically significant differences in the extent of adsorption losses of anthocyanins in clarified wines were found only between doses of 0.5 and 1.5 g/l (Figs. 6 and 7).

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Vliv čiření bentonitem na koncentraci anthokyaninů a barevnou intenzitu červených a růžových vín

ABSTRAKT: Cílem bylo sledovat koncentrační ztráty veškerých anthokyaninů červených a růžových vín v důsledku čiření různými dávkami dvou obchodních druhů bentonitů a analyticky i senzoricky zhodnotit rozdíly v intenzitě barvy čiřených a nečiřených vín. Ztráty anthokyaninů rostly se stupňovitým zvyšováním čiřicích dávek bentonitů, ale pouze mezi dávkami 0.5 a 1.5 g/l byly nalezeny statisticky významné rozdíly. Pro velikost změn intenzity barvy nebyl podstatný typ experimentálního bentonitu, ale jeho čiřicí dávka. Senzorickým hodnocením barevné intenzity bylo prokázáno, že u růžových vín dávka 0.5 g/l a u červených vín dávka 1.5 g/l statisticky významně snížila intenzitu zabarvení vzhledem k nečiřeným vzorkům.

Klíčová slova: víno; čiření; bentonit; veškeré anthokyaniny; barevná intenzita

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