

The Effect of Berries Extract Addition on the Phenolic Content and on the Colour of Rosé and White Wines

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*The influence of different concentrations of *Vaccinium vitis-idaea* respectively *Vaccinium myrtillus* fruits extracts on the colour and on the phenolic composition of rosé and white wines was evaluated during 2 months of storage. The measurement of the color of the wines was carried out using the Glories methods. The chromatic Glories parameters are: color intensity, tonality, percentage of yellow, percentage of red and percentage of blue. These chromatic characteristics may help us to better understand the contribution of berries extracts to the overall wine colour.*

Keywords: phenolic composition, Glories methods, chromatic characteristics, berries extracts

In the last years there is a considerable interest in the development of food colorants derived from natural sources for the replacement of synthetic food colorants. The role of anthocyanins as food colouring agents becomes very important since they form the reds and the blues of many fruits and vegetables, and provide the attractive colour of many fruit juices, wines, jams and preserves [1, 2].

Wines are intensively studied because of their continuous changes in composition during storage. Phenolic compounds constitute one of the most important quality parameters of wines since they contribute to their organoleptic characteristics, particularly color, astringency and bitterness. During wine maturation and ageing, phenolic compounds participate in numerous chemical reactions. Anthocyanins are progressively transformed into more stable oligomeric and polymeric pigments which give rise to important changes in the colour (from bright-red to brick-red hues) [3, 4]. The color of anthocyanins can be stabilized and enhanced by copigmentation reactions. Copigmentation can be a valuable, natural tool for improving the color of anthocyanin rich food products, the color of which can be stabilized and enhanced by the addition of different plant extracts rich in copigments. In

wines, instability and reactivity of anthocyanins, together with copigmentation reactions, are thought to be responsible for the changing colour of aging wines [5].

The knowledge of chromatic characteristics of wines is especially important because this field is a quality criterion both for the young wine and for the wine submitted to maturation and ageing. The necessity of defining the chromatic characteristics of wines lead to several methods of analysis, the most frequently used being the spectrometric methods [6].

The purpose of this study was to evaluate the influence of two natural extracts, *Vaccinium vitis-idaea* fruits (cranberry) extract which contain 60.18 mg total polyphenols (GAE)/mL, respectively *Vaccinium myrtillus* fruits (bilberry) extract with 61.34 mg GAE/mL, on the colour properties and the phenolic content of rosé and white wine during 2 months.

Experimental part

Materials and methods

Preparation of samples

We used bottled wine, purchased from a supermarket. The wines analyzed were Rosé (2005 and 2006) and Riesling (2006), Recca^o, Romania.

No sample	Type -year	Type of extract added	mL extract / L wine
1.	Rosé - 2006	<i>Vaccinium vitis-idaea</i> fruits extract	10
2.	Rosé - 2006	<i>Vaccinium vitis-idaea</i> fruits extract	5
3.	Rosé - 2006	<i>Vaccinium myrtillus</i> fruits extract	10
4.	Rosé - 2006	<i>Vaccinium myrtillus</i> fruits extract	5
5.	Rosé - 2005	<i>Vaccinium vitis-idaea</i> fruits extract	10
6.	Rosé - 2005	<i>Vaccinium vitis-idaea</i> fruits extract	5
7.	Rosé - 2005	<i>Vaccinium myrtillus</i> fruits extract	10
8.	Rosé - 2005	<i>Vaccinium myrtillus</i> fruits extract	5
9.	Riesling - 2006	<i>Vaccinium vitis-idaea</i> fruits extract	10
10.	Riesling - 2006	<i>Vaccinium myrtillus</i> fruits extract	10
11.	Riesling - 2006	<i>Vaccinium vitis-idaea</i> fruits extract	5
12.	Riesling - 2006	<i>Vaccinium myrtillus</i> fruits extract	5

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The work has been carried out on a set of 12 samples prepared as following:

We added the extract into the wine samples by injecting it through the cork avoiding the contact with oxygen. During the experiment, the samples were kept in darkness at a temperature of about 15 °C.

Analytical determinations

Total anthocyanins determination

For the determination of total anthocyanins content, a sample of 1 mL was taken from each sample of wine and diluted to a volume of 10 mL with acidified alcoholic solution (20 cm³ of conc. HCl, d = 1.19 g/cm³ diluted to a volume of 1000 cm³ with EtOH 96%). The absorbance of each sample was measured at 550 nm. Total anthocyanins content were calculated using the following equation [7]:

$$m = 0.015 \cdot D_0 \quad (\text{mg/mL})$$

where: D_0 - absorbance of sample at 550 nm;
0,015 - the experimental coefficient.

The determination of total polyphenols content

The content of total phenolic compounds in wine samples was determined by the Folin-Ciocalteu's (FC) reagent, using gallic acid as standard. This method is based on the reduction of a phosphowolframite-phosphomolybdate complex by phenolics to blue reaction products. For the preparation of the calibration curve, 1 mL aliquots of 5, 10, 20, 25 and 50 mg/L aqueous gallic acid solutions were mixed with 0.50 mL FC reagent. Concerning the wine, to 1 mL of wine sample, 2 mL of 20% carbonate solution and 0.5 mL of FC reagent were added. The absorbance of the standards and samples was measured at 765 nm after 30 min of reaction at room temperature. The results were expressed as mg of gallic acid equivalents (GAE)/L. All determinations were performed in triplicate [8, 9].

Determination of the chromatic characteristics

The determination of chromatic characteristics using Glories method was carried out on the undiluted wine (by diluting the wine suffers modifications regarding the balance between the colored compounds) [10, 11].

Colour density of wine samples was determined by measuring the absorbance at 420, 520 and 620 nm in a

quartz cell of 1 cm path length, using a spectrophotometer. For colour-related parameters a direct measurement of absorbance (A) of the wines samples was carried out. The chromatic *Glories* parameters are colour intensity (I.C.*), tonality, percentage of yellow, percentage of red and percentage of blue and were calculated using the following equations:

$$I.C.* = A_{420} + A_{520} + A_{620}; \quad T = \frac{A_{420}}{A_{520}}$$

420% = $\frac{A_{420}}{I.C.*} \cdot 100$ where 420% represents the contribution of yellow to the colour of the wine

520% = $\frac{A_{520}}{I.C.*} \cdot 100$ where 520% represents the contribution of red to the colour of the wine

620% = $\frac{A_{620}}{I.C.*} \cdot 100$ where 620% represents the contribution of blue to the colour of the wine

The Glories method is widely known and more frequently used by oenologists.

Results and discussions

Global phenolic determinations, usually performed in wineries, have provided useful information in relation to the evolution of wine polyphenols during ageing in bottle. The changes registered in the phenolic content during ageing in bottle are expected to affect the wine color in different ways.

The results obtained using Glories method for the determination of chromatic characteristics and the content of anthocyanins respectively the total polyphenols of the samples analyzed are presented in tables 1- 4. In order to emphasize the effect the berries extracts had on the composition, respectively on the colour of the wines, the reference samples of the three wines were also analyzed (table 1).

The wine samples containing the berries extract were analyzed during 2 months. Tables 2, 3 and 4 show the results obtained after one day (t_0), respectively after 45 days ($t_0 + 45$ days). We observed that after 45 days the values of the parameters remain unchanged.

Table 1

THE COMPOSITION, INTENSITY AND TONALITY OF THE COLOR OF THE WINES ANALYZED

Parameters analyzed	Rosé 2005		Rosé 2006		Riesling 2006	
	t_0	$t_0 + 45$ days	t_0	$t_0 + 45$ days	t_0	$t_0 + 45$ days
Total anthocyanins (mg/L)	238	236	239	235	174	170
Total polyphenols (mg gallic acid /L)	290.64	288.72	303.40	301.42	235.45	232.17
Colour intensity	0.68	0.56	0.64	0.50	0.08	0.03
Tonality	1.06	0.96	0.98	0.96	2.61	2.54

Table 2

THE COMPOSITION, INTENSITY AND TONALITY OF THE COLOR OF THE ROSÉ WINES (2005) ANALYZED AFTER 45 DAYS FROM THE ADDING OF THE BERRIES EXTRACT

Parameters analyzed	Rosé + 10 g/L EM*		Rosé + 5 g/L EM*		Rosé + 10 g/L EA*		Rosé + 5 g/L EA*	
	t_0	$t_0 + 45$ days	t_0	$t_0 + 45$ days	t_0	$t_0 + 45$ days	t_0	$t_0 + 45$ days
Total anthocyanins (mg/L)	295	240	252	239	382	264	290	240
Total polyphenols (mg gallic acid /L)	432.82	335.76	417.02	321.36	460.52	334.35	461.06	319.74
Colour intensity	0.70	0.60	0.69	0.59	0.85	0.73	0.79	0.68
Tonality	1.07	1.06	1.07	1.05	0.99	0.98	1.02	1.01

*EM = *Vaccinium vitis-idaea* fruits extract; EA = *Vaccinium myrtillus* fruits extract

Table 3
THE COMPOSITION, INTENSITY AND TONALITY OF THE COLOR OF THE ROSÉ WINES (2006) ANALYZED AFTER 45 DAYS FROM THE ADDING OF THE BERRIES EXTRACT

Parameters analyzed	Rosé + 10 g/L EM		Rosé + 5 g/L EM		Rosé + 10 g/L EA		Rosé + 5 g/L EA	
	t ₀	t ₀ + 45 days	t ₀	t ₀ + 45 days	t ₀	t ₀ + 45 days	t ₀	t ₀ + 45 days
Total anthocyanins (mg/L)	310	248	241	238	332	286	272	240
Total polyphenols (mg gallic acid /L)	469.71	357.61	386.50	244.76	573.70	473.93	480.75	389.21
Colour intensity	0.65	0.53	0.65	0.53	0.72	0.60	0.68	0.56
Tonality	1.11	1.08	1.11	1.08	1.06	1.01	1.04	1.04

*EM = *Vaccinium vitis-idaea* fruits extract; EA = *Vaccinium myrtillus* fruits extract

Table 4
THE COMPOSITION, INTENSITY AND TONALITY OF THE COLOR OF THE WHITE WINES (2006) ANALYZED AFTER 45 DAYS FROM THE ADDING OF THE BERRIES EXTRACT

Parameters analyzed	Riesling + 10 g/L EM		Riesling + 5 g/L EM		Riesling + 10 g/L EA		Riesling + 5 g/L EA	
	t ₀	t ₀ + 45 days	t ₀	t ₀ + 45 days	t ₀	t ₀ + 45 days	t ₀	t ₀ + 45 days
Total anthocyanins (mg/L)	182	174	178	172	319	205	307	192
Total polyphenols (mg gallic acid /L)	256.01	237.18	242.16	236.42	259.47	247.24	257.96	246.27
Colour intensity	0.16	0.07	0.13	0.05	0.25	0.08	0.27	0.13
Tonality	2.13	2.89	2.60	2.65	1.39	1.37	1.48	1.48

*EM = *Vaccinium vitis-idaea* fruits extract; EA = *Vaccinium myrtillus* fruits extract

During the storage of the wines (without the supplementary adding of colorants) the intensity of the colour decreases [12]. The natural colorants extracted from the berries enrich the colour of rosé wine immediately after the adding of the extract and maintain this characteristic even during the storage. The most intense colour was noticed for the rosé of 2005 at the beginning of the experiment. The intensity of the color for the rosé of 2005 was with 4% higher than the intensity of the rosé of 2006 and with 60% higher than the white wine.

By adding fruit extract of *Vaccinium vitis-idaea* in concentration of 10 g/L (w/v) respectively 5g/L (w/v) to the rosé samples, their color intensity increases with 1-2% (initial intensity of samples without extract was 0.68 (rosé 2005) and 0.64 (rosé 2006) at a value of intensity 0.70 respectively 0.69 (rosé 2005 with extract added) and 0.65 (rosé 2006 whit extract added) in the first day) compared to the increase of 10-15% registered in the first day for the rosé samples which contained fruit extract of *Vaccinium myrtillus* in the same amounts. For the samples containing bilberry extract, the intensity of colour at the end of the experiment was at least 5% higher than the intensity of wine without fruit extract.

The 2006 wine had slightly higher values of tonality, and also, slightly higher percentage of yellow while the red tones percentage decreases (fig. 1). However, though all the wines samples showed very low value of tonality, the differences were not significant at a visual test. At the same time, an accumulation of anthocyanins was observed with no significant differences between the wines samples from 2005 and 2006.

White wine is given less importance than red wine, as it contains lower quantities of polyphenols. The contents in anthocyanins determine the significant and eye observable difference between the white and rosé wines. The intensity of colour for the white wine which contains berries extract increases significantly in the first days and maintains at higher values compared to the reference sample until the end of the experiment. Once the berries

extract was added we can notice a slight browning of the colour as a result of the phenols oxidation to quinones which can polymerize to form macromolecules of yellowish-brown colour. This is more obvious for the samples which contain bilberries extract, whose color is time persistent brick-reddish.

When using the sum of absorbances registered at 420, 520, and 620 nm as a measure of colour intensity, any compound that contributes to an increase in „browness“ could be considered as factor of color change of the wine.

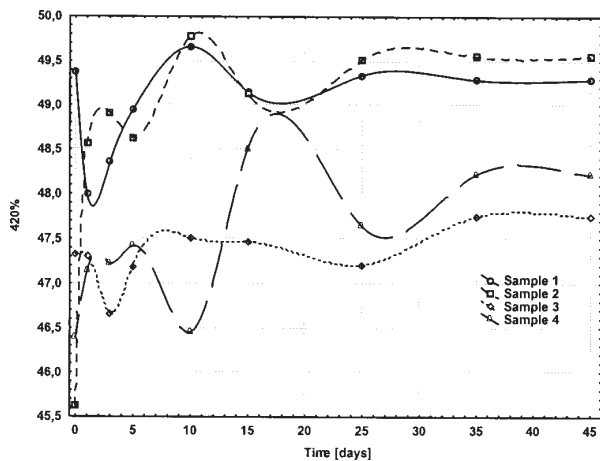
The contributions of the natural extracts added to the final colour of the rosé and white wine, expressed by the variation of the three colours: yellow, red and blue in time are illustrated in figures 1, 2 and 3.

The adding of the fruits extracts has a direct influence towards the intensity of the color. We can notice from figure 1 that the cranberry extract contributes to the enrichment in yellow of the colour of wine, while the bilberry extract enriches in red the colour of wine, proportional to the quantity of extract added.

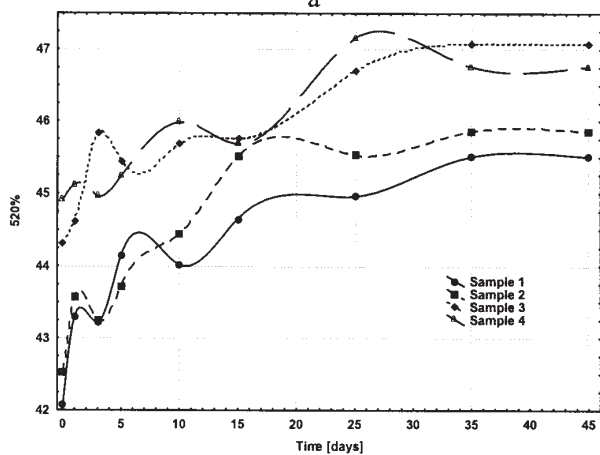
Due to the fact that around 520 nm the absorbance of the samples is stronger, the predominant color perceived is red; we can observe also the yellowish-brown component under the brick-reddish hue, and the blue component which expresses itself as a darker slightly bluish hue.

We can observe a 2-3% greater contribution of the yellow color compared to the red to the final colour of the rosé wines depending on the year of production (figs. 1, 2). The contribution of the blue colour to the final colour of wine has the highest value for the 2006 productions of wine. The higher value of the blue component of these wines is probably due to a good co-pigmentation or condensation of the anthocyanins with other polyphenolic compounds, giving rise to newly formed anthocyanins which stabilize the violet tonalities of the wine.

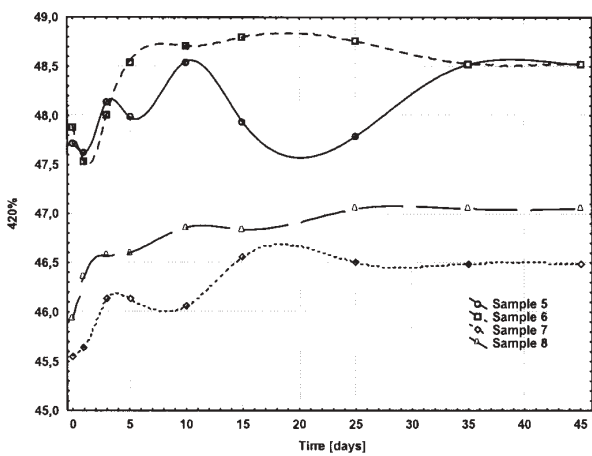
From the data obtained we can notice that a percentage of maximum 40% of the red contribution to the colour of white wine, the final colour of the wine is brick-reddish



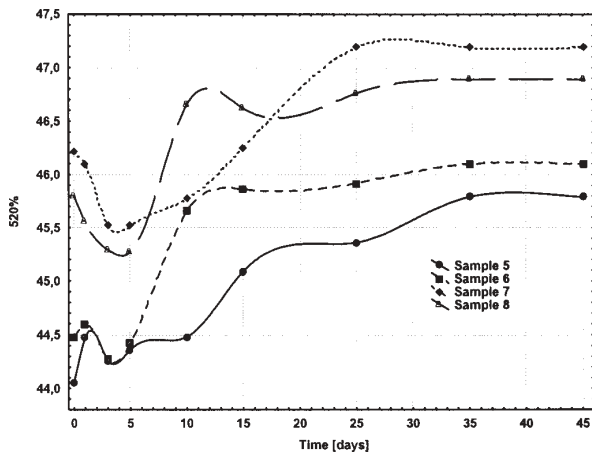
a



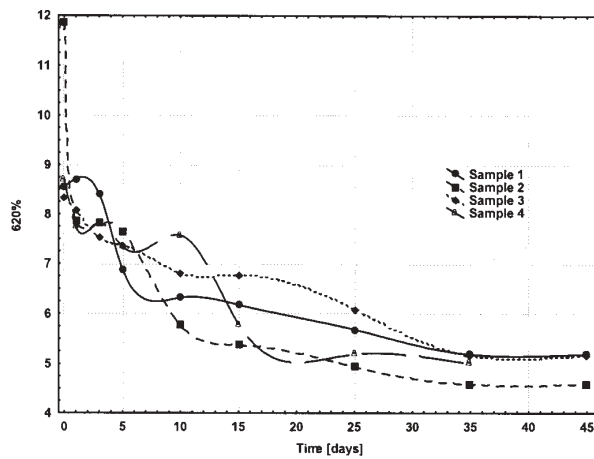
b



a

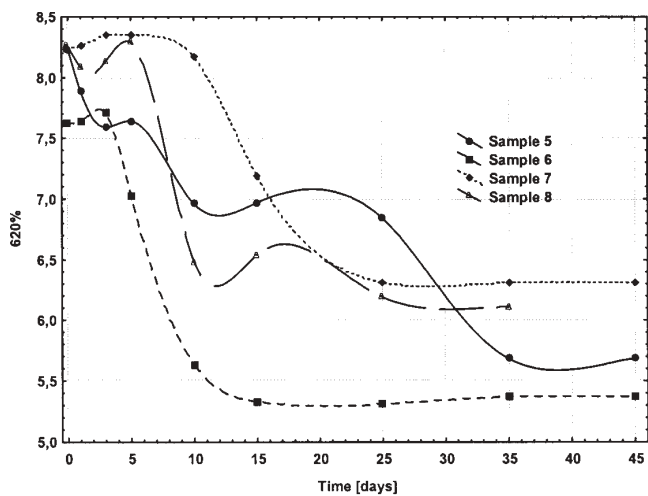


b



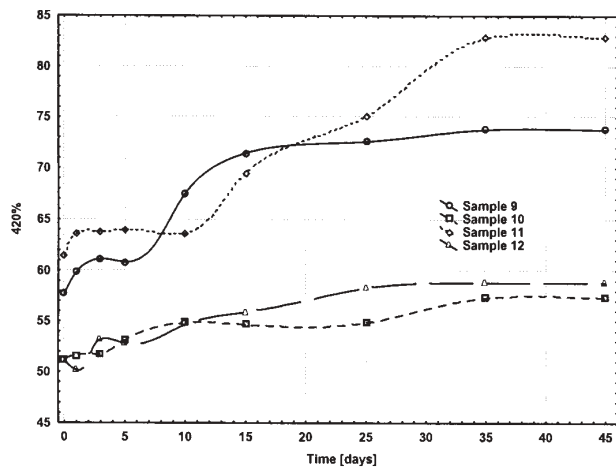
c

Fig. 1. The variation of the chromatic characteristics in time for 2006 rosé wine in the presence of fruit extracts. a) variation of the chromatic characteristic 420% - yellow; b) variation of the chromatic characteristic 520% - red; c) variation of the chromatic characteristic 620% - blue

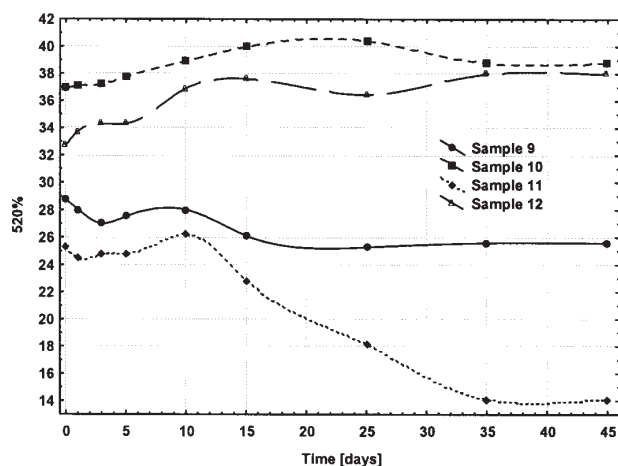


c

Fig. 2. The variation of the chromatic characteristics in time for 2005 rosé wine in the presence of fruit extracts. a) variation of the chromatic characteristic 420% - yellow; b) variation of the chromatic characteristic 520% - red; c) variation of the chromatic characteristic 620% - blue



a



b

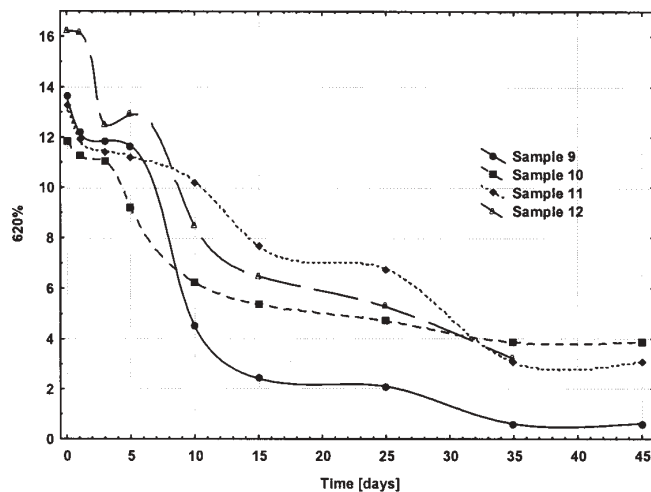
(samples 10, 12 are enriched with bilberry extract). The samples enriched with cranberry extract (samples 9, 11) have a final orange hue, over 55% of the final colour of the wine being given by yellow. This statement is sustained by literature [2] which mentions that for a pH lower than 3, the colour of anthocyanins (which exist as flavilium cations) is orange or red (the pH of the white wine analyzed is 2.97).

Conclusions

Maintaining a strong and stable color in wines is problematic during processing and storage. In this study it was shown that the colour of rosé and white wines could be enhanced by berries extracts. This enhancement is most probably due to the overall increment of anthocyanin content of wines, in the case of berries extracts addition, but also other substances of the extracts, like other flavonoids and phenolic acids, may take part in the colour enhancement reactions.

The establishment of the chromatic characteristics of rosé and white wines were carried out using Glories method. The results obtained lead to the conclusion that wine is in a continuous modification regarding its composition during the storage and the colour of wine depends on the stability of the phenolic compounds in time.

Best color stability was obtained by using *Vaccinium myrtillus* extract as a color enhancer, as the colour intensity



c

Fig. 3. The variation of the chromatic characteristics in time for 2006 white wine in the presence of fruit extracts.

- a) variation of the chromatic characteristic 420% - yellow;
 b) variation of the chromatic characteristic 520% - red;
 c) variation of the chromatic characteristic 620% - blue

of wine enhanced with *Vaccinium myrtillus* extract decreased the least during storage.

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