The effect of bud density on the composition of colour in red wines

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Summary: Authors investigated the effect of fruit load on the composition of colour substances and of *reservatrol* in red wines of some grape varieties grown in Hungary. The content of both, anthocyanin compounds and reservatrol showed practically the same responses to fruit load and the same profile in all the varieties studied. Determination of these compounds solely is not sufficient to identify the respective grape variety.

Introduction

The quality of the grapes is influenced mainly by the effect of the variety, the growing site and the vintage. Among those factors the vintage can not be controlled, virtually, whereas the site and the choice of variety are subject to economical considerations, so we are constrained to compromises. The quality depends considerably on the yield per area. On the one hand, yield is genetically limited; on the other hand it is modified by the structure of plantation, i.e. the rootstock, the training system and the spacing of rows and plants. The most important factor of building up quality is yield, which in turn is modified also by fitotechnical means.

Investigation was the objective of several attempts of researchers aiming to find out the causal relations between yield and quality especially of the colour composition in the wine produced (*Guidoni & Schubert*, 2000; *De La Hera Orts* et al., 2000). Albeit the compounds originating from grape go through different physico-chemical and biochemical changes, significant difference could be noticed between components of wines in plantations, which had different yields.

The yield is regulated efficiently by cultivation technology, first of all by pruning. So the number of buds, which are permitted to grow shoots on the stocks, limits fruit load. Practically, the grape quality of every variety decreases with the increasing fruit load beyond a certain level. Declining quality is expressed in a lower brix grade, increasing acid content, moreover, in unfavourably changes of acid compounds. Similarly, quality decreases also to a certain degree if pruning was too heavy. That means low bud densities may break down the productive balance. The fruit load becomes unusually low, leaf area per unit of yield increases causing self-shading especially in those varieties which are known to grow thick foliage. Generally, red grapes react more sensitively than white grape varieties, as in the former we have to expect changes in polyphenolic compounds, especially in the quantity of colour substances.

In the present publication results of four years in a long-term experiment are reported. The effect of fruit load has been checked in three different red grape varieties. The bulk of data available prove unambiguously that yield and wine quality depend on the level fruit load. Among components of quality the composition of polyphenols has been stressed in wine samples of the year 1999. The different anthocyanines, as well as the ratio of acylised and not acylised derivatives are important from the point of view of colour stability. It is a known fact that the anthocyanin composition of different varieties is typical causing the characteristic colour of the respective variety. We would like to demonstrate by the proofs presented how much pruning as a viticultural technique may change the composition of colour in red wines. We also examined the formation of resveratrol, a phytoalexin, which was found also in other plant families (e.g. stilbenes) having beneficial physiological effect on heart and circulatory diseases. The occurrence of that compound among others has been related to the level of fruit load.

Material and method

The information gathered on the experimental plantations is reported in *Table 1*.

There are four levels of fruit load examined in the experiment:

load 0: 2 buds/m^2 (one half long shoot /stock)
load 1: 3.8 buds/m^2 (one whole long shoot/stock)
load 2: 7.6 buds/m^2 (two whole long shoots/stock)
load 3: 11.4 buds/m^2 (three whole long shoots/stock)

Table 1 – Characterisation of the plantations

Variety & clone	Growing site	Category of quality of the site	Year of planting	Area per vinestock (m²)	Type of training
Kékfrankos M6/44 Kõlyuktetõ	Eger First class	1989	3.15	umbrella	
Kékoporto Kt1 Dobrányi dülõ	Eger First class	1988	2.97	umbrella	
Pinot noir Öreg-hegy	Kerecsend First class	1982	3.15	umbrella	
Loads: see the text					

Each level of load was represented in three replications with 10–15 stocks per plot randomly distributed in the plantation. The viticultural data were processed according to the three replications, the vinicultural data according to the levels of fruit load. The assessment of vine-stocks and indices of fertility supplemented the phytotechnical data. During the growing season the photosynthetic activity and indices of leaf area were determined. Yield and mean mass of bunches were registered as well as density and acidity of must obtained at the moment of vintage.

Preparation of the wine samples

In the vintage of 1999, 20 kg clean fruit samples were weighed of each of the blue grape varieties: *Kékfrankos*, *Kékoporto* and *Pinot noir*. The squashed berries were fermented on husk after having been inoculated with selected yeast. The mash was pressed on about the 12th day and 100 mg/l SO_2 was added to the juice. At cellar temperature (12–14 °C) the samples were drawn off after 8–10 days and stored in glass balloons filled up completely.

Determination of the colour substances

The components of anthocyanines were identified by HPLC techniques according to a modified method of *Waterhous* et al. (1994). Its advantage is that no preparation of the samples is needed.

The device:	HP 1050 HPLC
Detector:	UV (diode series)
Column:	ODS Hypersil C–18 (100 mm)
λ	520 nm
T:	25 °C
flow:	0.5 ml/min
Separation:	by gradient elution

Eluents:

Solution A:	$NH_4H_2PO_4$ 50 mMol, pH=2.6
Solution B:	Solution A 20% acetonitril 80%
Solution C:	H_3PO_4 0.2 Mol adjusted to pH=1.5 by NH_4OH

Program of gradients expressed in minutes

Solution A	Solution B %	Solution C %
0	8	92
20	20	80
25	30	70
30	40	60
35	80	20
(100 % Solution A) 40	0	0

To the identification of the components the standards of FLUKA & ROTH Ltd were used comparing the maxima of their absorption (A_{max}) as displayed in *Table 2*.

Table 2 – Absorption maxima of the anthocyanin components

Compound	Amax (nm)
delphidinin-3-monoglycoside	520
cyanidin-3-monoglycoside	520
petunidin-3-monoglycoside	520
peonidin-3-monoglycoside	512–514
malvidin-3-monoglycoside	522
cyanidin-3-glycoside-acetate	514–516
petunidin-3-glycoside-acetate	528
peonidin-3-glycoside-acetate	516
malvidin-3-glycoside-acetate	528
peonidin-3-glycoside-(p-cumarate)	522
malvidin-3-glycoside-(p-cumarate)	534
Waterhaus et al. (1994)	

Determination of the isomeres of Reservatrol

Reservatrol isomeres, the physiologically active substances, are determined by the procedure of *Kállay–Török* (1997) by injection HPLC method. The decisive characters from the point of view of vinification are the tint of colour, colour intensity, the conent of total polyphenols, catechin, anthocyanin, leuco-anthocyanin, which are treated by usual spectro-photometry, whereas content of alcohol and extracts by picnometer.

Results and conslusions

Most interest of the many data available are assigned to the composition of colour, which are presented as wine characteristics of the respective grape varieties *(Tables 3, 4, 5)*.

Table 3 – Yield and parameters of wine quality of the variety Kékfrankos (vintage 1999)

Bud load (buds/m²)	Must density (Mmo)	Yield (t/ha)	Colour intensity	Colour tint	Total of polyphenol (mg/l)	Catechin (mg/l)	Leuco anthocyanin (mg/l)
2.0	19.6	0.57	1.90	0.94	901	289	763
3.8	19.4	0.58	2.14	0.91	1003	318	714
7.6	18.4	1.38	2.63	0.82	866	238	667
11.4	18.7	1.30	3.05	0.62	944	274	709

Table 4 – Yield and parameters of wine quality of the variety Kékoporto (vintage 1999)

Bud load (buds/m²)	Must density (Mmo)	Yield (t/ha)	Colour intensity	Colour tint	Total of polyphenol (mg/l)	Catechin (mg/l)	Leuco anthocyanin (mg/l)
2.0	19.8	0.26	3.09	0.93	1298	589	541
3.8	20.8	0.97	3.65	0.97	1319	748	792
7.6	20.2	1.23	3.62	0.93	1291	642	852
11.4	21.0	1.38	2.98	0.94	1115	477	465

Table 5 – Yield and parameters of wine quality of the variety Pinot noir (vintage 1999)

Bud load (buds/m²)	Must density (Mmo)	Yield (t/ha)	Colour intensity	Colour tint	Total of polyphenol (mg/l)	Catechin (mg/l)	Leuco anthocyanin (mg/l)
3.8	19.1	0.98	1.22	0.88	758	332	611
7.6	19.6	1.47	1.28	0.80	810	387	640
11.4	20.0	1.71	1.08	0.91	756	391	422

Results are published in *Table 6, 7* and *8/a*, and more easily to be visualised in *Figures 1* to *7*.

Table 6 – Effect of bud load on the composition of colour substances (Kékfrankos)

Compound (mg/l) in MD	load 0	load 1	load 2	load 3
delphidinin-3-monoglycoside	1.85	2.23	0.00	1.68
cyanidin-3-monoglycoside	0.00	0.00	0.00	0.00
petunidin-3-monoglycoside	5.46	5.09	3.00	6.53
peonidin-3-monoglycoside	7.33	9.28	1.35	2.75
malvidin-3-monoglycoside	136.23	127.96	83.42	130.57
cyanidin-3-glycoside-acetate	0.00	0.00	0.00	0.00
petunidin-3-glycoside-acetate	0.00	0.99	1.66	1.83
peonidin-3-glycoside-acetate	0.00	0.00	1.03	0.43
malvidin-3-glycoside-acetate	4.75	0.00	3.11	6.91
peonidin-3-glycoside-(p-cumarate)	0.00	0.00	0.00	0.00
malvidin-3-glycoside-(p-cumarate)	2.52	3.04	4.13	4.72

MD: malvidin-3,5-diglycoside

Table 6/a – Effect of bud load on resveratrol and on some colour compounds (Kékfrankos, 1999)

	Total anthocyanins mg/l	Σ of acylated compounds %	$V_{Ac/Cu}$	Cis-resveratrol mg/l	Trans-resveratrol mg/l
load 0	158.14	4.50	1.88	3.45	1.85
load 1	148.59	2.70	0.33	2.44	1.86
load 2	97.70	10.16	1.40	2.50	1.74
load 3	155.42	8.94	1.94	2.31	1.60

Σ acylated compounds % means the ratio of acylated compounds related to the concentration of all anthocyanins

$V_{Ac/Cu}$ means the ratio of anthocyanin.glycoside-acetates and cumarates

Table 7 – Effect of bud load on the composition of colour substances (Kékoporto)

compound (mg/l) in MD	load 0	load 2	load 3
delphidinin-3-monoglycoside	8.20	3.13	3.71
cyanidin-3-monoglycoside	0.00	0.00	0.00
petunidin-3-monoglycoside	16.06	7.32	10.90
peonidin-3-monoglycoside	7.28	3.62	3.87
malvidin-3-monoglycoside	143.93	111.94	111.16
cyanidin-3-glycoside-acetate	0.22	0.00	0.00
petunidin-3-glycoside-acetate	6.21	1.67	1.62
peonidin-3-glycoside-acetate	2.04	0.00	0.00
malvidin-3-glycoside-acetate	18.11	13.67	13.61
peonidin-3-glycoside-(p-cumarate)	0.00	0.00	0.00
malvidin-3-glycoside-(p-cumarate)	4.98	7.48	6.22

MD: malvidin-3,5-diglycoside

Table 7/a – Effect of bud load on resveratrol and on some colour compounds (Kékoporto, 1999)

	Total anthocyanins mg/l	Σ of acylated compounds %	$V_{Ac/Cu}$	Cis-resveratrol mg/l	Trans-resveratrol mg/l
load 0	207.03	15.27	5.34	1.81	0.84
load 2	148.83	15.33	2.05	1.89	1.26
load 3	151.09	14.20	2.45	1.52	1.08

Σ acylated compounds % means the ratio of acylated compounds related to the concentration of all anthocyanins
$V_{Ac/Cu}$ means the ratio of anthocyanin.glycoside-acetates and cumarates

Table 8–

Compound (mg/l) in MD	load 0	load 2	load 3
delphidinin-3-monoglycoside	0.00	0.00	0.00
cyanidin-3-monoglycoside	0.00	0.00	0.00
petunidin-3-monoglycoside	0.00	0.00	0.17
peonidin-3-monoglycoside	0.00	0.97	2.65
malvidin-3-monoglycoside	56.37	34.48	57.95
cyanidin-3-glycoside-acetate	0.00	0.00	0.00
petunidin-3-glycoside-acetate	0.00	0.00	0.74
peonidin-3-glycoside-acetate	0.00	0.00	0.00
malvidin-3-glycoside-acetate	0.00	0.00	0.00
peonidin-3-glycoside-(p-cumarate)	0.00	0.00	0.00
malvidin-3-glycoside-(p-cumarate)	1.64	0.18	0.00

MD: malvidin-3,5-diglycoside

Table 8/a –

	Total anthocyanins mg/l	Σ of acylated compounds %	$V_{Ac/Cu}$	Cis-resveratrol mg/l	Trans-resveratrol mg/l
load 0	58.01	2.83	0.00	3.28	1.07
load 2	35.63	0.52	0.00	2.72	1.27
load 3	61.54	1.20	0.00	3.29	1.05

Σ acylated compounds % means the ratio of acylated compounds related to the concentration of all anthocyanins
$V_{Ac/Cu}$ means the ratio of anthocyanin.glycoside-acetates and cumarates
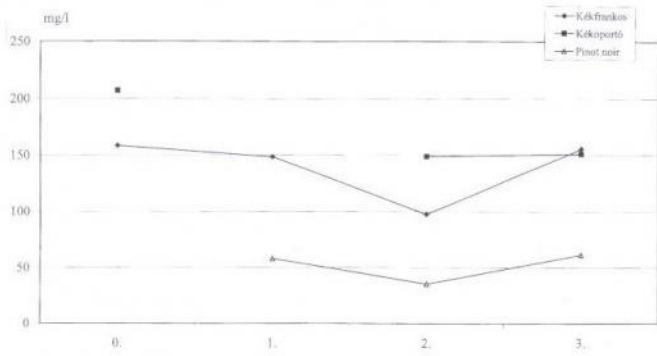
Figure 1 – Changes in the concentration of total anthocyanins as an function of bud load
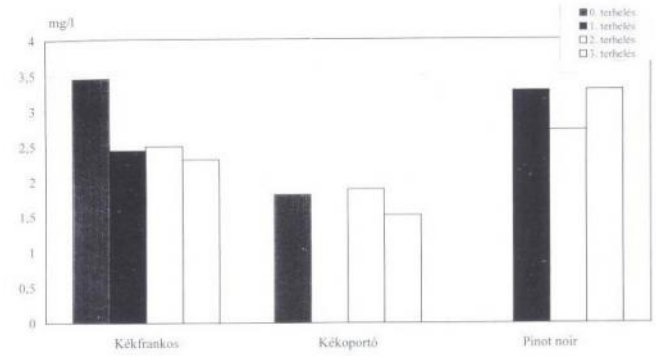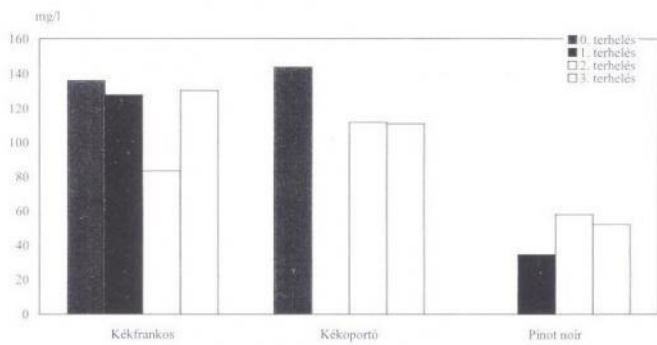X = bud load (0, 1, 2, 3)   Y = mg/l

Figure 2 – Changes in the concentration of malvidin-3-monoglycoside as an function of bud load
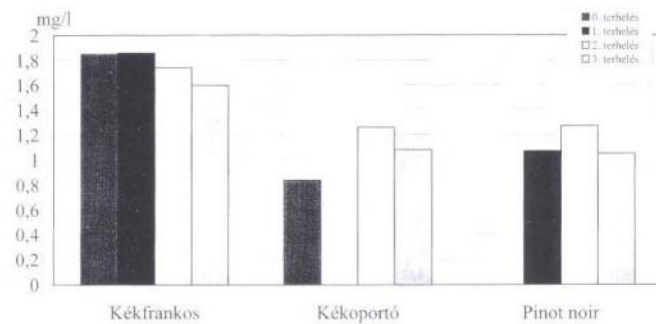0. terhelés = load 0   1. terhelés = load 1   stb.

Figure 3 – The ratio (%) of acylated compounds as a function of bud load
X = bud load (0, 1, 2, 3)   Y = mg/l

Figure 4 – Changes in the ratio of glycoside-acetates and glycoside-cumarates as a function of bud load
X = bud load (0, 1, 2, 3)   Y = mg/l

Figure 5 – Changes in the concentration of cis-reversol as a function of bud load
0. terhelés = load 0   1. terhelés = load 1

Figure 6 – Changes in the concentration of trans-reversol as a function of bud load
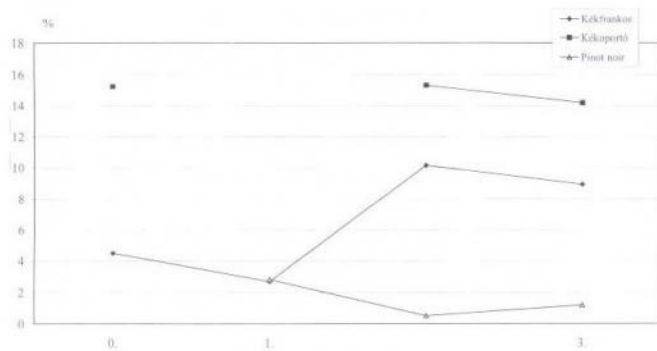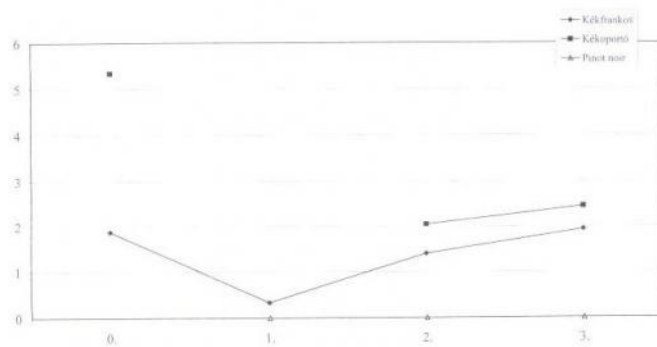0. terhelés = load 0   1. terhelés = load 1

1. delfinidin-3-monoglükozid
2. cianidin-3-monoglükozid
3. petunidin-3-monoglükozid
4. peonidin-3-monoglükozid
5. malvidin-3-monoglükozid
6. cianidin-3-glükozid-acetát
7. petunidin-3-glükozid-acetát
8. peonidin-3-glükozid-acetát
9. malvidin-3-glükozid-acetát
10. peonidin-3-glükozid-(p-kumarát)
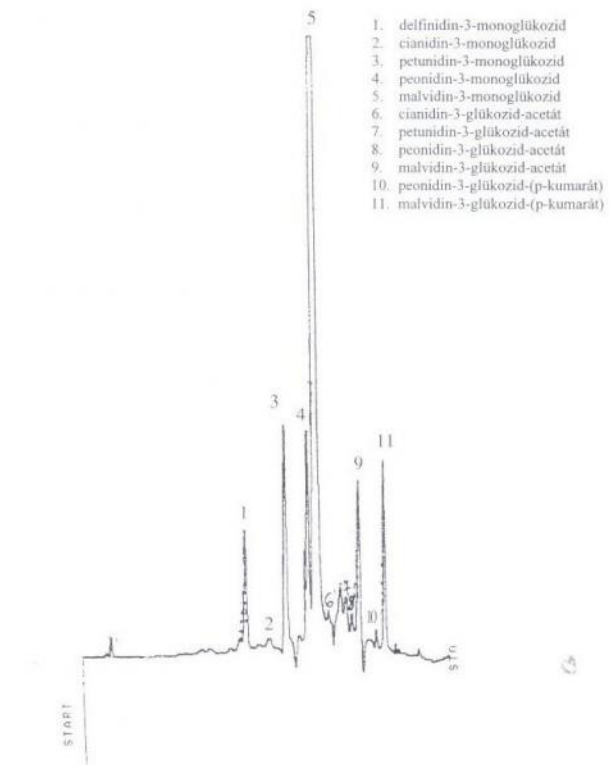11. malvidin-3-glükozid-(p-kumarát)

Figure 7 – Composition of colour substances in the wine of Kékfrankos (at bud load 0)

All of the three varieties observed prove the effect of the load of buds on the composition of colour substances, included also the formation of acylated compounds, which may turn out to be specific on the variety.

Some parameters, which are considered as characteristic of the variety (Σac%, Vac/Cu) are equally functions of the fruit load.

Among the varieties the wine of *Pinot noir* contains only a minimum of acylated derivatives.

The concentration of both the trans- as well as the cis-reservatrol depends on fruit load. By purposeful viticultural technologies the yield of reservatrol concentration is susceptible to be increased.

Generally, it should be stated that the colour composition of a red wine is not sufficient to infer immediately to the identity of the respective variety.

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