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Cross-Correlation of Quality Parameters of Musts and Wines Enriched with Lignans

Pavla NOVOTNÁ[1], Jan TŘÍSKA[2], Pavel HÍC[3], Josef BALÍK[3], Naděžda VRCHOTOVÁ[2], Jan STROHALM[1] and Milan HOUŠKA[1]

[1]Food Research Institute Prague, Prague, Czech Republic; [2]Global Change Research Centre, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; [3]Mendel University in Brno, Faculty of Horticulture, Lednice, Czech Republic

Abstract

Novotná P., Tříska J., Híc P., Balík J., Vrchotová N., Strohalm J., Houška M. (2016): **Cross-correlation of quality parameters of musts and wines enriched with lignans.** Czech J. Food Sci., 34: 24–31.

Hydroxymatairesinol (HMR) is the main lignan found in spruce knots. This lignan has been used for enrichment of musts and wines. Quality parameters of these products have been studied for several years and for storage times up to one year. Parameters included HMR concentration, antioxidant activities expressed as ferric reducing antioxidant power and 2,2-difenyl-1-picrylhydrazyl, total polyphenols, and sensory parameters, i.e. consumer acceptability. The main goal of this work was to study and provide relationships between the above mentioned quality parameters. We analysed cross-correlations of all these parameters and found statistically significant correlations between lignan concentration and consumer acceptability, which can be phrased as a warning against high lignan concentrations. The strongest correlations were found between antioxidant parameters and total polyphenol content that supports the antioxidative behaviour.

Keywords: hydroxymatairesinol (HMR); enrichment of must and wine; correlations; antioxidant activity; total polyphenol concentration; consumer acceptability

Nomenclature: DPPH – 2,2-difenyl-1-picrylhydrazyl (mmol Trolox/l); FRAP – ferric reducing antioxidant power (mmol Trolox/l); lignan CONI – α-conidendrin (mg/l); lignan HMR – hydroxymatairesinol (mg/l or μg/ml); lignans concentration HMR+CONI (mg/l or μg/ml); N – number of experimental data (–); p – number of parameters of correlation equation (–); R_{crit} – critical value of correlation coefficient (–); total polyphenols (mg/l expressed as gallic acid)

Wine, especially red wine, contains a lot of healthy components, e.g. antioxidants, phenolics, and anthocyanins (Lachman *et al.* 2009). In addition to these components we can also find a small content of lignans.

Lignans belong to the group of chemical compounds called plant phenols and they have revealed a variety of biological effects over the last two decades. In terms of the structure, lignans are composed of two phenyl propane units that are connected via the central beta carbon atoms of two side chains; it is thus the most common dimer. In recent years various types of softwoods have been described that have high concentrations of lignans in the form of oligolignans. We can find lignans in wood, cereals, trees, roots, plant leaves, and seeds. Baderschneider and Winterhalter (2001) discovered lignans together with other components in Riesling wine. Nurmi *et al.* (2003) published a screening study of the content of lignans in selected wines. Marchal *et al.* (2015) published recently how the oak lignans can influence the wine gustatory features.

Lignans excel as antioxidant, anticancer, antiviral, antibacterial, insecticidal, fungicidal, and estrogenic compounds as well as being protective against heart diseases (Slanina 2000; Harmatha 2005; Yatkin *et al.* 2014). Recently, Laavola *et al.* (2015) published

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on the effects of Scots pine (*Pinus sylvestris*) phenolic compounds, which have anti-inflammatory properties.

Lignans and other plant polyphenols can ordinarily be found in the heartwood of trees. Lignans are especially common in conifers and flavonoids can be found in wood with a hard core. Recently, a group of Finnish scientists published an important discovery regarding the richest natural source of lignans. Holmbom *et al.* (2003) found lignans, about 5–10% by mass, in tree knots. It is interesting that Norway spruce (*Picea abies*) contains up to 6–29% lignans by mass, with the most abundant lignan being hydroxymatairesinol (HMR), which accounts for 70–85% of the total lignan content.

Kangas *et al*. (2002) presented a paper on the antioxidant and antitumor effects of HMR. This is very important information and provides the basis for food enrichment with lignans. HMR was approved by the US FDA in 2004 as a dietary supplement and since 2006 it has been marketed under the brand name HMRlignan™. There are several other dietary supplements based on lignans, e.g. Life-Three HMR lignans, femMED Breast Health, Prostate Support Formula, and Enterolactone Enhancer. Our own experiments discovered that knots from Norway spruce (*Picea abies*) trees, grown in Czech highland forests, are a rich source of HMR (Balík *et al*. 2015a). Based on this discovery we have studied methods for enriching various sorts of wine and wine musts with HMR lignan and then predicted various quality parameters for those products: content of HMR, antioxidant activity, total polyphenol content, and total consumer acceptability. Wines with alcohol were enriched by dipping chips from spruce tree knots into the wine. Musts, without alcohol, were enriched by adding a small amount of HMR alcohol extract.

The main goal of this work was to evaluate the above-mentioned quality parameters after the addition of lignans. Cross-correlations between all studied parameters from wine and must beverages were periodically analysed over a storage period that lasted about one year.

MATERIAL AND METHODS

The details of sample preparation and the descriptions of experiments were presented in papers by Balík *et al.* (2015b) and Novotná *et al.* (2015).

Sample preparations. Wines, with increased natural lignan content, were produced by adding ground spruce knots corresponding to at least 10 mg lignan/l of beverage during the technological phase of training or maturation. We used Grüner Veltliner wine and Blauer Limberger (Blaufränkisch) grape varieties for wine preparation. The process continued with 14 days of maceration at 15–20°C, combined with decanting and filtration. An alternative procedure was also used for wines or wine-based beverages to increase the natural content of lignans. During the technological phase of training or aging, a liquid extract of spruce knots (corresponding to at least 10–30 mg of lignan in one litre of beverage) was added with vigorous stirring at 25–30°C.

For non-alcoholic grape must we prepared lignans extracted in alcohol. We used hexane to remove the terpenes and resin compounds. Then the processed chips were added to the alcohol solution and lignans were extracted to reach the desired concentration of HMR. The content of HMR was 91.63 g/l of the alcoholic extract and the content of alpha-conidendrin (CONI) was 7.25 g/l of alcoholic extract.

If the goal was to prepare a must with increased natural lignan content, we added an alcohol extract of spruce knots to the must at 25–30°C with vigorous stirring. The extract was added to produce lignan content between 10 and 50 mg/l of beverage, while keeping the alcohol content below 0.5% by volume. The modified must was microbially stabilised using heat pasteurisation. Another technology for producing the must with an increased content of natural lignans involves adding ground knots to the must with the goal of increasing the lignan content to 10–50 mg/l of beverage. Before grinding, the knots were pretreated with hexane to remove terpenes and resin compounds. The must was then heated to 80°C and cooled. Afterwards the product was separated from the milled spruce knots by decanting, centrifugation, or filtration. The final product was microbially stabilised using heat pasteurisation.

Sample storage. Prepared samples of wines were stored for 13 months and musts for 12 months in refrigerators, between 1 and 3°C. Samples of wines were analysed after 2, 6, and 13 months of storage and of musts after 1, 5, 9, and 12 months of storage.

Lignan content analysis. HMR was identified as the main lignan in the spruce knots used in our study. In addition, CONI was found in small concentrations. Analyses were based on the extraction of spruce knots in 96% ethanol. Before extraction, non-polar substances, mainly terpenes and resin compounds, were removed using hexane. Liquid

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chromatography with a diode array detector HP G1315B (DAD, Hewlett-Packard, Palo Alto, USA) was used for analysis. 7-hydroxymatairesinol concentrations were assessed using a Hewlett Packard 1050 with an Agilent G1315B diode array detector, and a Phenomenex Luna C18 (2) column (3 mm, 2 × 150 mm). Mobile phase: water–acetonitrile–*o*-phosphoric acid; mobile phase A 5% acetonitrile + 0.1% *o*-phosphoric acid; and mobile phase B 80% acetonitrile + 0.1% *o*-phosphoric acid. For separation, a gradient from 20% B to 80% B (within 20 min) was used, the flow rate was 0.25 ml/min and the column temperature was 25°C. HMR was detected at 220 nm. It is important to note that for a correlation analysis, the sum of HMR and CONI concentrations for each sample was used. Detection limit for both HMR and CONI using HPLC and DAD detection was 0.06 µg/ml with a correlation coefficient of 0.9903 for HMR and 0.9875 for CONI using five calibration points.

Determination of antioxidant activity using FRAP and DPPH methods. Determination using the ferric reducing antioxidant power (FRAP) method was done at a pH of 3.6, in acetate buffer (23 mM sodium acetate trihydrate in solution of 34 mM acetic acid). The reaction mixture contained 12 mM $FeCl_3$ solution + 10 mM 2,4,6-tris(2-pyridyl)-*s*-triazine in 40 mM HCl solution; the buffer was at a ratio of 1 : 1: 10. An amount of 2 ml of the reaction mixture was mixed with 25 µl of the diluted sample (diluted with deionised water) in a disposable plastic cuvette (10 mm) and, after 10 min, the resulting solution was measured using a Helios-beta spectrophotometer (Spectronic Unicam, Cambridge, UK) at a wavelength of 593 nm. The antioxidant activity was calculated from a Trolox standard calibration curve.

For the DPPH method, 1.9 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical solution in methanol (c = 0.1 mmol/l) was mixed with 0.1 ml of diluted sample (deionised water) in a disposable plastic cuvette (10 mm). After 30 min, the absorbance at 515 nm was measured using a Helios-beta spectrophotometer. The antioxidant activity was calculated from a Trolox standard calibration curve.

Total polyphenol determination using Folin-Ciocalteu reagent. In this method, 0.5 ml of white wine (or 0.1 ml of red wine) was put into a 50 ml volumetric flask with approximately 20 ml of deionised water and mixed with 1 ml of Folin-Ciocalteu reagent. The flask was shaken and, after 3 min, 5 ml of 20% Na_2CO_3 was added to the mixture. The flask was shaken thoroughly again and filled with deionised water. After standing for 30 min, the colour of the mixture was measured, in 10 mm cuvettes, at a wavelength of 700 nm, using a Helios-beta spectrophotometer (relative to a blank sample). The total polyphenol content was calculated from a gallic acid calibration curve.

Sensory assessment. Sensory assessments of the samples were produced using a graphical hundred-point scale and an ordinal method was used by a panel of ten selected sensory assessors. The members of the sensory panel consisted of 10 up 12 assessors selected according to the ISO standard. A graphical hundred-point scale for sensory analysis was chosen purposely to use observed data for a linear cross-correlation between all studied parameters. The samples were stored in a refrigerator which kept samples at the same temperature for the extended period of testing used in our technique. For purposes of this work, we selected the most important parameter of the sensory quality evaluation titled "consumer acceptability".

Statistical methods. All analytical parameters were measured in two trials, with the exception of the sensory evaluation (there were 6 to 12 assessors). We calculated the arithmetic mean and standard deviation of all parameters. These mean values, valid for different storage times, were used for a linear cross-correlation between all studied parameters. Calculated values of each correlation coefficient were compared with critical values of correlation coefficients given in standard tables (Štěpánek 1975) and valid for a degree of freedom (number of experimental data N – number of parameters of correlation equation p = 2). Statistically significant correlations were predicted only in cases where the calculated correlation parameter was greater than the critical value of the correlation parameter valid for a given degree of freedom.

RESULTS AND DISCUSSION

White and red wine enriched with lignans from ground spruce knots (chips). Correlation coefficients of all parameters from white and red wines enriched with lignans (by addition of ground spruce knots = chips) are given in Table 1. It is apparent that only the parameters of consumer acceptability and the FRAP antioxidant activity were significantly correlated for white wines and there is no significant correlation between any of the measured parameters for red wines.

Correlation coefficients of all parameters from all data valid for white and red wines enriched with lignans are given in Table 2.

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Table 1. Correlation coefficients of white and red wine with chips

	White wine with chips				Red wine with chips			
	lignans	FRAP	DPPH	total polyphenols	lignans	FRAP	DPPH	total polyphenols
Consumer acceptability	0.017	**0.386**	0.036	0.116	0.337	0.270	0.116	0.000
Lignans		0.302	0.109	0.259		0.439	0.192	0.417
FRAP			0.275	0.238			0.010	0.302
DPPH				0.116				0.101

R_{crit} for 27 data points = 0.381; R_{crit} for 18 data points = 0.468 (correlation with lignans); in bold significant correlation

Table 2. Correlation coefficients of white and red wine with chips – all data together

	Lignans	FRAP	DPPH	Total polyphenols
Consumer acceptability	0.160	0.223	0.178	0.159
Lignans		0.092	0.231	0.152
FRAP			**0.937**	**0.966**
DPPH				**0.956**

R_{crit} for 54 data points = 0.264; R_{crit} for 36 data points = 0.319 (correlation with lignans); in bold significant correlation

It is apparent that strong correlations were found between the two different parameters of antioxidant activity (FRAP and DPPH) and the total polyphenol content. Statistically significant correlations are presented in Figure 1.

White and red wine enriched with lignans through the addition of an alcohol extract made from ground spruce knots. Correlation coefficients of all parameters from white and red wines are given in Table 3, and for all wine samples in Table 4. There was no significant correlation between the measured

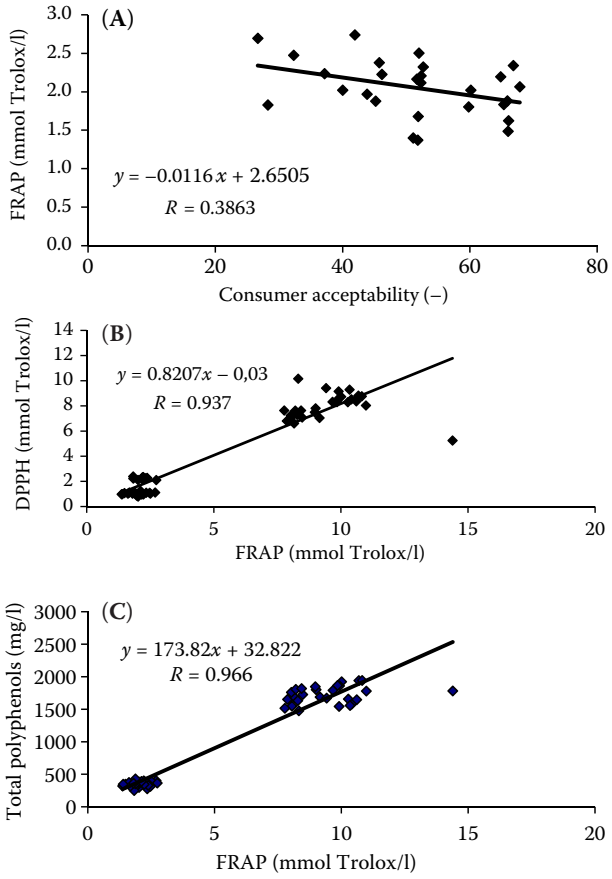

Figure 1. FRAP parameter vs consumer acceptability of white wine with chips (**A**), DPPH vs FRAP (**B**), and total polyphenols vs FRAP (**C**) of white and red wine with chips

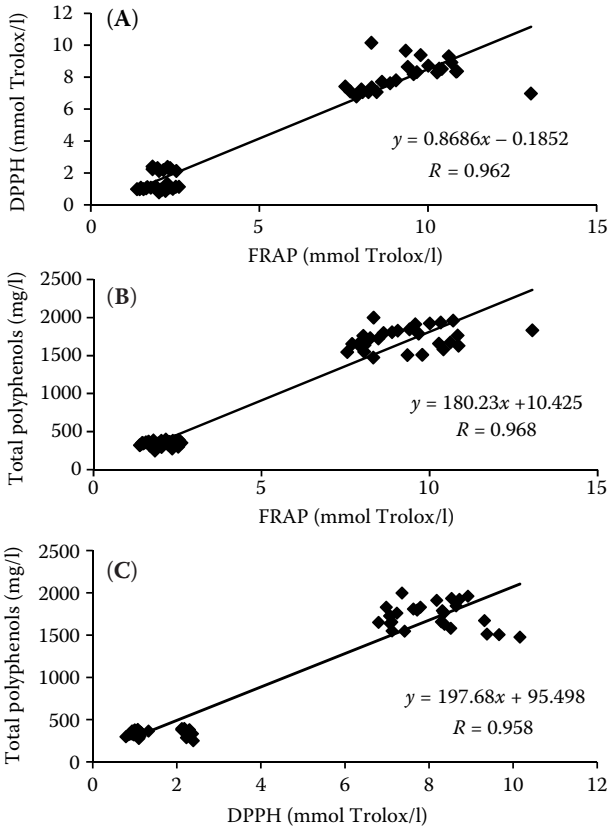

Figure 2. DPPH vs FRAP (**A**), total polyphenols vs FRAP (**B**), and total polyphenols vs DPPH (**C**) of white and red wine with extract

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Table 3. Correlation coefficients of white and red wine with extract

	White wine with extract				Red wine with extract			
	lignans	FRAP	DPPH	total polyphenols	lignans	FRAP	DPPH	total polyphenols
Consumer acceptability	0.303	0.171	0.195	0.244	0.116	0.232	0.328	0.107
Lignans		0.382	0.001	0.391		0.223	0.145	0.260
FRAP			0.232	0.141			0.361	0.269
DPPH				0.047				0.191

R_{crit} for 27 data points = 0.381; R_{crit} for 18 data points = 0.468 (correlation with lignans)

parameters. On the other hand, Table 4 contains correlation coefficients for all data, valid for both types of wine, and statistically significant values are visible. Namely, significant correlation coefficients were found between FRAP and DPPH antioxidant activities, consumer acceptability and antioxidant parameters FRAP, DPPH, and total polyphenol content. There was also a significant correlation between both antioxidant parameters and total polyphenol content. Correlations with statistically significant correlation coefficients are presented in Figures 2.

White and red must enriched with lignans from ground spruce knots. Correlation coefficients of all parameters from white and red musts are presented in Table 5, and for all must samples in Table 6. There were statistically significant correlations between consumer acceptability and antioxidant activity DPPH and total polyphenol concentration and also a strong correlation between DPPH and FRAP and antioxidant parameters (DPPH, FRAP) and total polyphenol concentration for white must with chips.

Table 4. Correlation coefficients of white and red wine with extract – all data together

	Lignans	FRAP	DPPH	Total polyphenols
Consumer acceptability	0.136	**0.329**	**0.344**	**0.274**
Lignans		0.142	0.219	0.169
FRAP			**0.962**	**0.968**
DPPH				**0.958**

R_{crit} for 54 data points = 0.264; R_{crit} for 36 data points = 0.319 (correlation with lignans); in bold significant correlation

The consumer acceptability is highly correlated with lignan content, the correlation also evident for FRAP and DPPH, and total polyphenols correlated with antioxidant parameters FRAP and DPPH for red must with chips.

Table 6 presents surprising evidence that all tested parameters correlated with each other and had statistical significance. Selected correlations with statistically significant correlation coefficients are presented

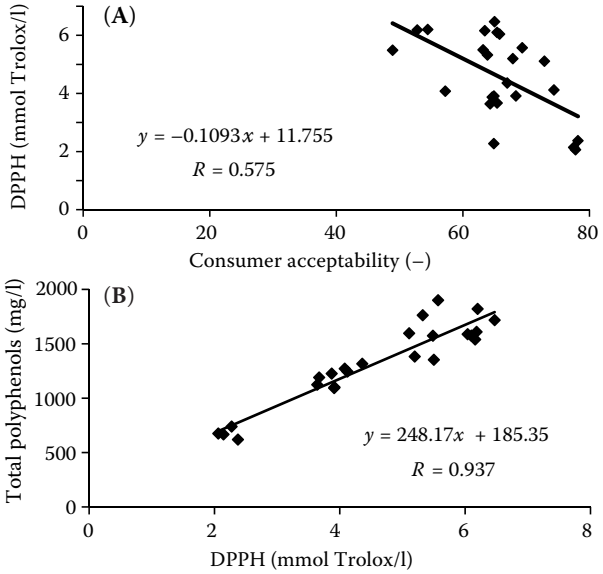

Figure 3. DPPH vs consumer acceptability (**A**) and total polyphenols vs DPPH (**B**)of white must with chips

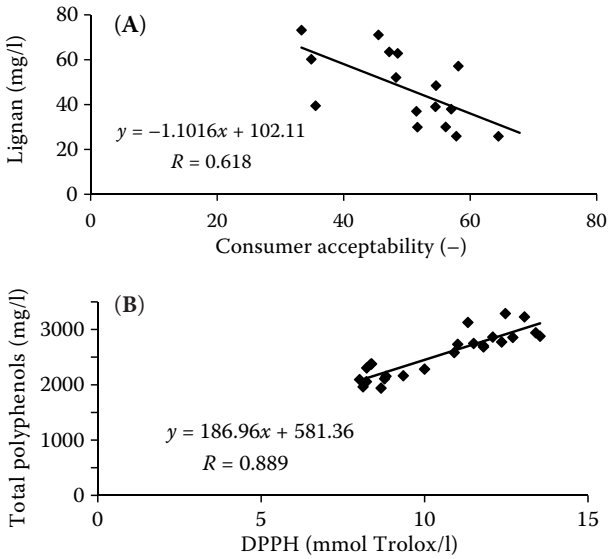

Figure 4. Lignans vs consumer acceptability (**A**) and total polyphenols vs DPPH (**B**) of red must with chips

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Table 5. Correlation coefficients of white must with chips and red must with chips

	White must with chips				Red must with chips			
	lignans	FRAP	DPPH	total polyphenols	lignans	FRAP	DPPH	total polyphenols
Consumer acceptability	0.483	0.397	**0.575**	**0.552**	**0.618**	0.076	0.026	0.042
Lignans		0.135	0.154	0.136		0.035	0.146	0.258
FRAP			**0.876**	**0.771**			**0.843**	**0.636**
DPPH				**0.937**				**0.889**

R_{crit} for 24 data points = 0.406; R_{crit} for 16 data points = 0.497 (correlation with lignans); in bold significant correlation

Table 6. Correlation coefficients of white and red musts with chips

	Lignans	FRAP	DPPH	Total polyphenols
Consumer acceptability	**0.791**	**0.506**	**0.562**	**0.570**
Lignans		**0.636**	**0.661**	**0.693**
FRAP			**0.964**	**0.916**
DPPH				**0.974**

R_{crit} for 48 data points = 0.279; R_{crit} for 32 data points = 0.349 (correlation with lignans); in bold significant correlation

in Figures 3–5. It can be seen that there are very strong relationships between parameters characterising white and red musts. The strongest correlations were found for all sample parameters without differentiation with regard to white or red grapes.

White and red must enriched with lignans through the addition of an alcohol extract made from ground spruce knots. Correlation coefficients of all parameters for white musts and red musts are given in Table 7 and for all must samples in Table 8.

It can be seen from Table 7 that there was a statistically significant correlation between white must (with lignan extract) and consumer acceptability. The correlation was negative and indicate that lower lignan concentrations had better consumer acceptability. It confirmed an assumption that the lignan concentration has to be calculated carefully with respect to consumer sensory assessment. Table 7 also shows excellent correlations between the antioxidant parameters FRAP and DPPH. The same correlation coefficient levels were predicted for total polyphenol content and antioxidant parameters. Table 7, valid for red must (with extract), shows very high correlation coefficients for consumer acceptability and antioxidant parameters FRAP, DPPH, and total polyphenol content. Excellent correlations were also

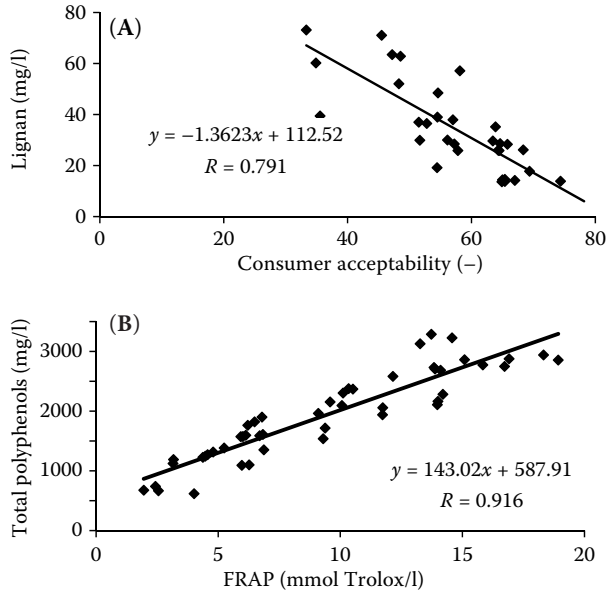

Figure 5. Lignans vs consumer acceptability (**A**) and total polyphenols vs FRAP (**B**) of white and red musts with chips

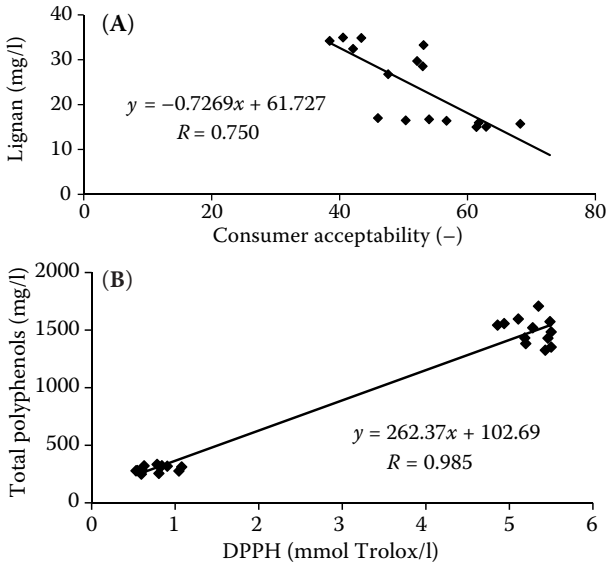

Figure 6. Lignans vs consumer acceptability (**A**) and total polyphenols vs DPPH (**B**) of white must with extract

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Table 7. Correlation coefficients of white and red must with extract

	White must with extract				Red must with extract			
	lignans	FRAP	DPPH	total polyphenols	lignans	FRAP	DPPH	total polyphenols
Consumer acceptability	**0.750**	0.079	0.099	0.069	0.228	**0.482**	**0.486**	**0.473**
Lignans		0.136	0.196	0.181		0.035	0.022	0.004
FRAP			**0.988**	**0.976**			**0.990**	**0.981**
DPPH				**0.985**				**0.993**

R_{crit} for 24 data points = 0.406; R_{crit} for 16 data points = 0.497 (correlation with lignans); in bold significant correlation

Table 8. Correlation coefficients of white and red musts with extract

	Lignans	FRAP	DPPH	Total polyphenols
Consumer acceptability	**0.398**	0.188	0.207	0.200
Lignans		0.010	0.070	0.020
FRAP			**0.990**	**0.978**
DPPH				**0.991**

R_{crit} for 48 data points = 0.279; R_{crit} for 32 data points = 0.349 (correlation with lignans); in bold significant correlation

found between the antioxidant parameters FRAP and DPPH and total polyphenols. Table 8 shows a very high correlation between consumer acceptability and lignan concentration. High correlation coefficients were found between the antioxidant parameters FRAP, DPPH and total polyphenol contents. Selected correlations with statistically significant correlation coefficients are presented in Figures 6–8. Very strong relationships were found between parameters characterizing white and red musts. The strongest correlations were found for all antioxidant parameters DPPH, FRAP, and total polyphenol content. Some significant correlations between lignan content and consumer acceptability are presented in Figures 6A and 8A. The dependence is negative; i.e. lower lignan concentrations were associated with higher values of consumer acceptability.

Statistically significant correlations between all studied parameters represent a tool for the design of new wine or must products with substantially increased HMR and CONI content. This procedure generates potentially healthier products with regard to antioxidant and antitumor effects. Wine enrichment with lignans regardless of the method (added extract of lignans or direct extraction of lignans from added ground spruce

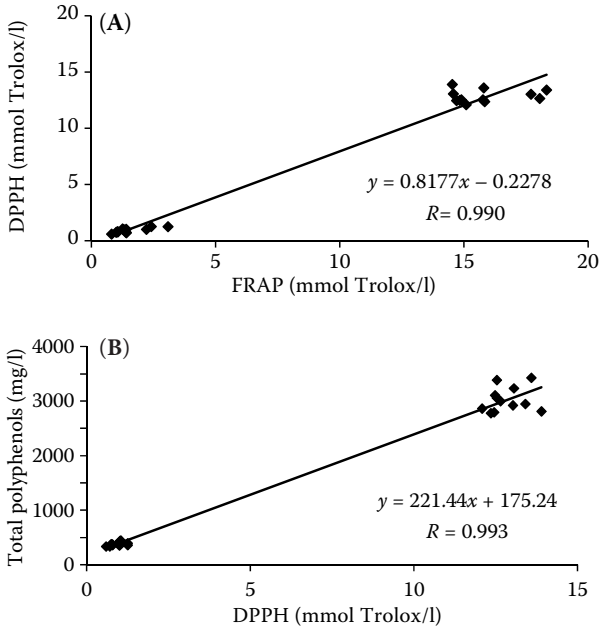

Figure 7. DPPH vs FRAP (**A**) and total polyphenols vs DPPH (**B**) of red must with extract

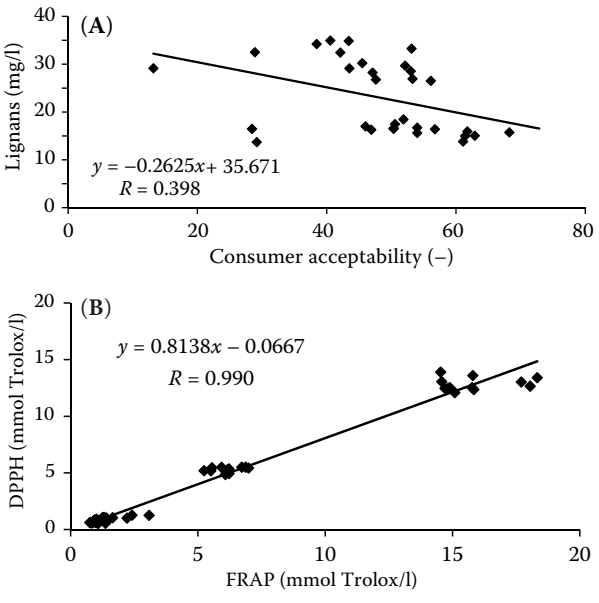

Figure 8. Lignans vs consumer acceptability (**A**) and DPPH vs FRAP (**B**) of white and red musts with extract

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knots) can influence the wine official title and has to be solved before wine commercialisation.

CONCLUSIONS

This work summarises all parameters characterising white and red wine and white and red musts enriched with HMR lignan obtained from ground spruce knots. In one group of products the ground spruce knots (chips) were dipped into the final product. In the other group the products were enriched by addition of an alcohol extract made from ground spruce knots. There were selected quality parameters such as lignan concentration, consumer acceptability, antioxidant characteristics FRAP and DPPH, and total polyphenol content. We analysed cross-correlations between all the above-mentioned parameters and found a variety of statistically significant correlations. One of the more important inverse correlations was found between lignan concentration and consumer acceptability. The correlation suggests that lignans have to be added judiciously in order to maintain consumer acceptability. The strongest correlations were found between antioxidant parameters with each other and total polyphenol content that supports this antioxidative behaviour.

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Corresponding author:

Ing. Milan Houška, CSc., Výzkumný ústav potravinářský Praha, Radiová 7, 102 31 Praha 10, Česká republika;
E-mail: m.houska@vupp.cz