

Wines with Increased Lignan Content by the Addition of Lignan Extracts

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Abstract

BALÍK J., HÍC P., KULICHOVÁ J., NOVOTNÁ P., TRÍSKA J., VRCHOTOVÁ N., STROHALM J., HOUŠKA M. (2016): **Wines with increased lignan content by the addition of lignan extracts.** Czech J. Food Sci., 34: 439–444.

Red and white wines [Grüner Veltliner white wine and Blue Limberger (Blaufränkisch) red wine (vintage 2013)] were enriched with lignan hydroxymatairesinol originated from spruce knots. These spruce knots with removed resin were extracted with ethyl alcohol of agricultural origin. Ethanol extracts of lignans were then used to enrich wine sorts. Enriched wines were stored for 13 months. At 2, 6, and 13 months, samples were taken and subjected to a variety of analyses and sensory evaluations. Analyses included 7-hydroxymatairesinol and alpha-conidendrin lignan content, antioxidant activity (as determined by FRAP), total polyphenols, and sensory evaluation. The obtained data were evaluated using the analysis of variance to determine which factors e.g. wine type, quantity of added lignan extracts, additional sugar, method of preservation, and storage time had the most significant influence on lignan content, antioxidant activity and polyphenol content. In all cases the lignan content in the wines was significantly influenced by the addition of lignan extracts. After one year of storage, lignan contents changed only moderately and added lignans were stable in stored wines. Total polyphenol content in wines and the antioxidant activity of wines were significantly influenced by the type of wine (i.e., red or white). The presented method of wine enrichment with lignans opens the door for the production of extra quality wines.

Keywords: enrichment of white and red wines; 7-hydroxymatairesinol; alpha-conidendrin; antioxidant activity; total polyphenol content; sensory evaluation

Nomenclature: FRAP – ferric reducing antioxidant power (mM Trolox/l); *F*-statistic – corresponding *F*-quantile (see table for analysis of variance for individual parameters); Lignan CONI – alpha-conidendrin; lignan HMR – 7-hydroxymatairesinol; lignan concentration HMR + CONI (mg/l); *P*-value – corresponding probability – if the *P*-value is lower than the selected significance level (the selected value is 0.05 here), this predictor is statistically significant (see the table for analysis of variance valid for individual factors); sensory evaluation (–); total polyphenols (mg/l expressed as gallic acid); importance – verbal expression of the results of analysis of variance (important/unimportant)

Lignans belong to a large group of plant phenols which have attracted our attention in the last two decades not only due to their multiple biological effects but also because of their structural abundance. From the structural viewpoint, plant lignans are formed by joining the two phenylpropanoid precursors at beta-carbon atoms of both propyl side chains. Most

frequently, they are dimers but also some higher oligolignans were described in UMEZAWA (2003) and WILFÖR *et al.* (2006). As secondary metabolites of vascular plants, lignans show also effects of antioxidants, antitumor protectants, antiviral, and antibacterial substances, insecticides, fungicides, oestrogens, antioestrogens and, last but not least,

substances protecting against cardiovascular diseases (THOMPSON *et al.* 1998; HARMATHA & DINAN 2003; LINDAHL *et al.* 2011).

Some plant lignans (matairesinol, secoisolariciresinol, lariciresinol, pinoresinol) can be metabolised by intestinal bacteria to the mammalian lignans enterodiol and enterolactone. Both enterolignans like oestrogens bind with low affinity to the oestrogen receptor. Metabolites of 7-hydroxymatairesinol (HMR) were characterised also as enterolactone and 7-hydroxyenterolactone by comparison with authentic reference compounds (HEINONEN *et al.* 2001; ADLERCREUTZ 2007).

Lignans are phytoestrogens present in seeds, vegetable oils, cereals, legumes, fruits, and vegetables as aglycones, glycosides, esterified glycosides, or as bio-oligomers. High levels of lignans were found out particularly in flax and sesame (MAZUR & ADLERCREUTZ 1998; MEAGHER & BEECHER 2000; SICILIA *et al.* 2003). The amount of lignans (lariciresinol, pinoresinol, secoisolariciresinol, matairesinol, syringaresinol, isolariciresinol) in red wines ranged from 0.812 mg/l to 1.406 mg/l. The main lignan in all studied wines was isolariciresinol and the percentage amount of mammalian lignan precursors varied from 34% to 43% in red wines (NURMI *et al.* 2003). The content of selected lignans (lariciresinol, pinoresinol, secoisolariciresinol, matairesinol) in white wines ranged from 0.155 mg/l to 0.255 mg/l. In grape juices, their common level is lower than 0.1 mg/l (MILDER *et al.* 2005).

The alternative rich source of plant lignans is a coniferous tree (*Picea abies*) and knots in trees have a potential for the extraction of e.g. HMR (SAARINEN *et al.* 2000; HOLMBOM *et al.* 2003). An application output of our own experiments was the national patent (BALÍK *et al.* 2015).

The objective of this research was to prepare lignan-enriched wine through the addition of lignan extracts from spruce knots (after resin removal) into the production process. During the period of 13 months the wine samples were analysed to determine the effects of the enrichment process, such as lignan content, antioxidant activity (using FRAP), and total polyphenol content after selected storage times. Sensory evaluations of wines were also performed. The secondary aim of our effort was to present a method how to develop a dietary supplement with enriched content of lignans of natural origin regardless of the wine law. The analysis of variance helped us to predict significant impacts of the preparation process parameters (addition of

lignan extracts, sugar addition, preservation method, heat treatment of grapes – thermomaceration, storage time) on analytical parameters (lignan content, antioxidant activity, total polyphenols, and FRAP).

MATERIAL AND METHODS

The methods mentioned here were described in detail by NOVOTNÁ *et al.* (2016). Therefore, only different parts are mentioned here.

Lignan extract preparation. The method used was the same as described by NOVOTNÁ *et al.* (2016). Knots of spruce (*Picea abies*) trees were milled using a cutting mill (Cutting Mill SM 100; Retsch, Haan, Germany) and extracted to gain the substantially concentrated alcoholic solution of HMR and alpha-conidendrin (CONI). The residual non-polar solvent was removed from the wooden material under reduced pressure using a vacuum rotary evaporator and a freeze dryer (Heto Power Dry PL3000; Thermo Fisher Scientific, San Jose, USA). Subsequently, the wood chips prepared in this way were used for making an extract using ethanol of agricultural origin with minimum alcoholic strength, 96% vol., at a boiling temperature under reflux. The content of HMR was 91.63 g HMR/l of the alcoholic lignan extracts and the content of alpha-conidendrin (CONI) was 7.25 g CONI/l of alcoholic lignan extracts.

Sample preparations. Grüner Veltliner dry white wine and Blue Limberger (Blaufränkisch) dry red wine (vintage 2013) were used. The liquid lignan extracts were added into the wines with vigorous stirring, at 25–30°C. The dosage of the liquid lignan extracts corresponded to the contents of lignan (HMR and CONI) in wine 15 and 30 mg/l of wine (samples E15 and E30, respectively). The lignan extracts were not added to the wine marked as sample E0. Sucrose was then added in an amount of 0 or 150 g/l and the wines were preserved using pasteurisation at 70°C for 15 min or by adding alcohol of agricultural origin to 18% vol.

Sample storage. Prepared samples of wines were stored at temperatures between 1 and 3°C for 13 months. Samples of wines were analysed after 2, 6, and 13 months of storage.

Lignan content analysis. HMR was identified as the main lignan in spruce knots. Alpha-conidendrin was also found in small concentrations.

Determination of antioxidant activity using the FRAP method. Determination using the FRAP

method was carried out at pH 3.6 in acetate buffer (23 mM sodium acetate trihydrate in a 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, and a buffer at a 1 : 1 : 10 ratio.

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 the Folin-Ciocalteu reagent.

Sensory assessment. Sensory evaluation of enriched wines was performed by a panel of trained evaluators. A line segment of 100 mm in length (equivalent to 100 points) was used, along which the evaluators marked the value of the parameter being tested. The number of the evaluators ranged from 9 to 12. The evaluators assessed the intensity of woody notes in the aroma of wines (0 very weak, 100 very strong), astringency intensity and bitterness of wines (0 very weak, 100 very strong), and their assessment of consumer acceptability of wines (0 unacceptable, 100 outstanding). Evaluation of all sample parameters was marked on the same straight line (differentiated by sample codes). Parameters valid for individual samples were evaluated by mean values and standard deviations.

Statistical methods. Significance of parameters of the preparation process (addition of lignan extracts, sugar addition, preservation method, heat treatment of grapes – thermomaceration, storage time) in relation to analytical parameters (lignan content, antioxidant activity, total polyphenols, and FRAP) was determined by analysis of variance. All analytical

parameters were measured using 2 samples with the exception of sensory evaluation, which was performed by 9 to 12 evaluators. Arithmetic mean and standard deviation were calculated for all parameters. These values were plotted in all figures (the column heights are averages and whisker or bar segments represent standard deviations). The statistical evaluation of the data was done using analysis of variance and QC Expert 3.1 statistical software (TriloByte Statistical Software, Pardubice, Czech Republic). The liquid lignan extracts were added into the wines with vigorous stirring, at 25–30°C. The dosage of the liquid lignan extracts corresponded to the contents of lignan (HMR and CONI) in wine 15 and 30 mg in 1 litre of wine (samples E15 and E30, respectively). The lignan extracts were not added to the wine marked as sample E0. Czech Republic). The following factors were evaluated: type of wine (red, white), quantity of added lignan extracts (E0, E15, or E30), quantity of added sugar (0 g; 150 g/l), preservation method (PA – pasteurisation, A18 – added alcohol to 18% vol.), and storage time (2, 6, and 13 months). Lignan content, antioxidant activity determined by FRAP, and polyphenol content were evaluated as variables that could affect the above-mentioned wine factors.

RESULTS AND DISCUSSION

Red and white wine with the addition of lignan extracts. As provided by ANOVA, the *F*-test for the influence of added lignan extracts (as predictor) on the content of lignans was highly significant ($P < 0.0001$). At the same time, the influence of wine type, sugar,

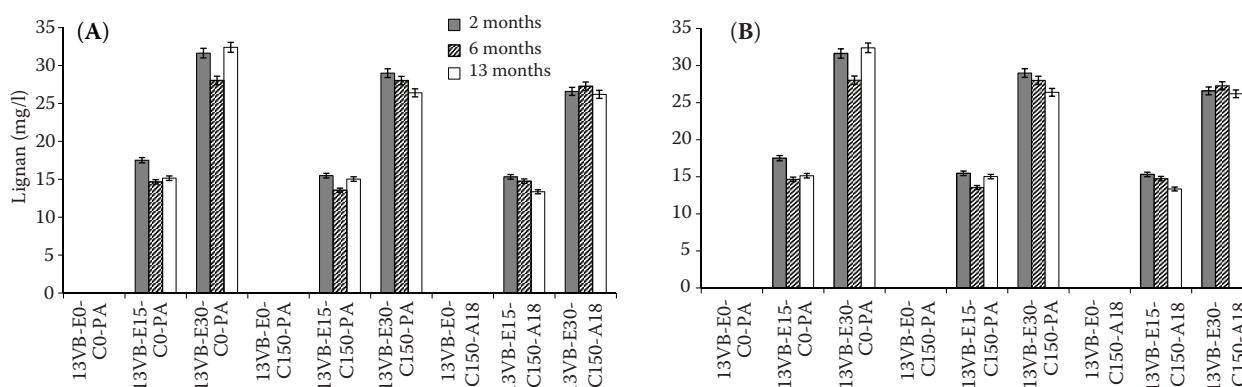


Figure 1. Lignan content in samples of (A) red and (B) white wine with added lignan extracts during storage. Explanation for Figures 1–4; 13 = year of test; VC = red wine, VB = white wine; E0, E15, E30 = lignan dose (HMR + CONI) per 0; 15; 30 mg/l; C0, C150 = sugar dose per 0 or 150 g/l; PA – pasteurisation (70°C, 15-min holding time); A18 – alcohol dose per 18% vol.; 2, 6, 13 – storage time in months (storage temperature 1–3°C)

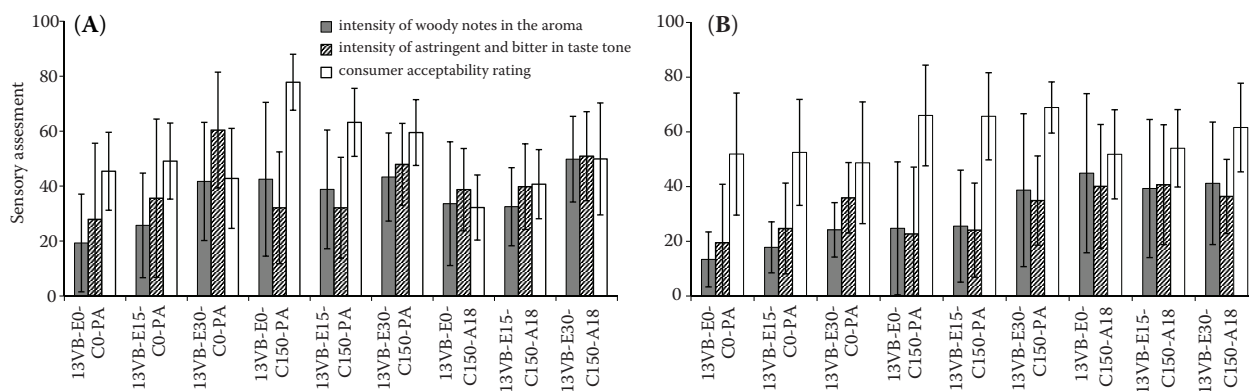


Figure 2. Sensory evaluation of (A) red and (B) white wine with added lignan extracts assessed after thirteen months of storage (number of evaluators = 10) (for explanation see Figure 1)

preservation, and storage time have no significant effect on the content of lignans. ANOVA for the influence of wine type (as predictor) on the content of total polyphenols and antioxidant activity showed highly significant F values ($P < 0.0001$). At the same time, the influence of added lignan extracts, sugar, preservation, and storage time on the same parameters was not significant.

Content of lignans. Determined lignan contents in wine samples after two months of storage correspond with the applied doses of lignan extracts (0; 15; 30 mg of lignans per 1 litre of wine). Samples with no added lignan extract designated by E0 were analysed and natural lignan contents were lower than the detection limit of the analytical method (Figure 1). There are data in literature and commercial leaflets about the recommended daily intake of 7-HMR lignan dose 40 mg per adult person per day [e.g. Swanson – <http://www.swansonvitamins.com/swanson-ultra-7-hmrlignans-from-norwegian-spruce-tree-40-mg-60-caps>].

Small differences were related to the technology of sample mixture with addition of sugar and alcohol.

After 6 months of storage, lignan contents in red and white wines mostly decreased in comparison with samples stored for 2 months. After 13 months of storage, lignan contents changed only moderately and added lignans were enough stable in stored wines. Samples prepared from Grüner Veltliner grapes had higher levels of lignans compared to samples prepared from Limberger grapes (Figure 1).

Sensory evaluation. All three groups of red wine samples showed an increase in the intensity of woody notes following the addition of lignan extracts (Figure 2A). There was an increase in the astringency intensity and bitterness in all three groups of red wines enriched with lignans; the highest values were associated with samples prepared with the dose of lignan extracts E30, but without sugar or alcohol supplement. Consumer acceptability for red wines with no added sugar (the first 3 samples) was not affected by the addition of lignan extracts. For wines with added sugar, a slight increase in the evaluation of consumer acceptability compared to the control sample was

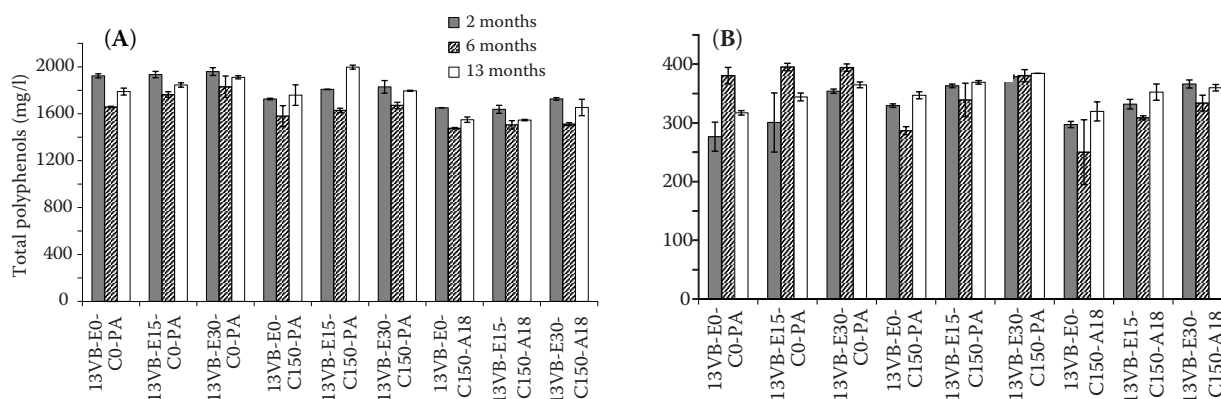


Figure 3. Total polyphenol content in samples of (A) red and (B) white wine with added lignan extracts during storage (for explanation see Figure 1)

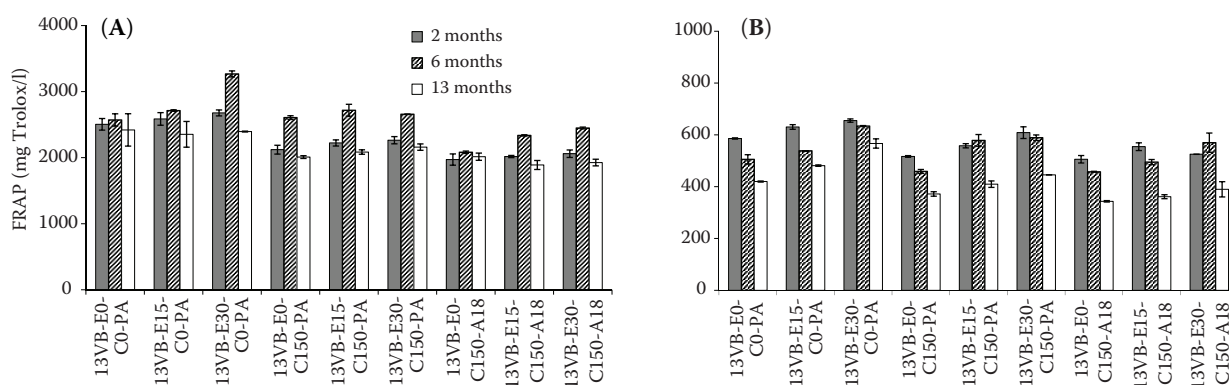


Figure 4. Antioxidant activity (FRAP) of (A) red and (B) white wine with added lignan extracts during storage (for explanation see Figure 1)

found. The sample of red wine without lignan extracts but with sugar addition had the best rating.

For white wines there was an increase in the woody aroma associated with the addition of lignan extracts regardless of the presence or absence of added sugar (Figure 2B). This was almost equally true of white wines prepared with sugar and alcohol. Astringency intensity and bitterness of white wines increased with the addition of lignan extracts, in both wines prepared with and without added sugar; for wines with added sugar and alcohol this parameter did not change. Consumer acceptability for all three white wine groups was little changed by the addition of lignan extracts. The greatest changes were associated with samples having been prepared with added sugar. The samples of white wine without addition of alcohol but with sugar addition had the best rating.

Total polyphenols. Total polyphenol content in all samples of red wine was about 5 times higher (from 1.480 mg/l to 1.910 mg/l) than for white wines (from 250 mg/l to 395 mg/l) irrespective of the addition of lignan extracts (Figure 3). The addition of lignan extracts had a higher effect on the contents of polyphenols in lignan-enriched white wines than in red wines. Polyphenol contents in lignan-enriched wines changed irregularly during storage. The lowest values were found in samples with added alcohol (Figure 3).

Antioxidant activity. Antioxidant activity of all samples of red wines determined by FRAP (from 7.55 mM Trolox/l to 13.06 mM Trolox/l) was about 4 times higher than that of white wines (from 1.37 mM Trolox/l to 2.62 mM Trolox/l) irrespective of the addition of lignan extracts (Figure 4). The antioxidant activity of red wines with added lignan extracts was generally highest after 2 months of storage, and then

declined during further storage. For white wines with added lignan extracts, antioxidant activity mostly decreased during storage and its values were significantly lower after 13 months (Figure 4).

CONCLUSIONS

This paper deals with the preparation of red and white wines enriched with lignans through the addition of lignan extracts from spruce knot chips stripped of resin. A sensory evaluation and analysis of lignans content, antioxidant activity and polyphenols was done for wines after 2, 6, and 13 months of storage. Determined lignan contents in wine samples agreed with the applied doses of lignan extracts. In the course of one-year storage no significant loss of lignan content was observed in the lignan-enriched red and white wines. The addition of lignan extracts did not significantly increase the total polyphenol content and antioxidant activity of wine samples. The intensity of woody aroma and also the astringency and bitterness intensity of all wine samples increased with the quantity of added lignan extracts. The best consumer acceptability rating of lignan-enriched wines was found for white wine samples with sugar addition (150 g/l). These doses seem to be optimum from sensory quality of the wine tested. The presented method of wine enrichment with lignans opens the door for production of wines or wine-based products of extra quality.

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