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Lignans in selected wines

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Abstract

Wines have been studied a lot and several different groups of phenolic compounds have been analysed in wine samples, but lignans in wines have been only preliminary studied. In this study, we analysed 10 different wines of which eight were red wines and two white wines. Samples were selected among the most consumed wines in Finland in year 1999. Eight different lignans were analysed with HPLC using coulometric electrode array detector. The amount of lignans in red wines ranged from 0.812 to 1.406 mg/l. The main lignan in all studied wines was isolariciresinol and percentage amount of mammalian lignan precursors varied from 34 to 43% in the red wines. The amount of lignans was much lower in the studied white wines compared with the red wines.
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Keywords: Wine; Lignans; HPLC

1. Introduction

Wines have been studied intensively and over 200 phenolic compounds with possible biological activities have been identified (German, 1998). Several studies have been carried out to quantify the amount of flavonols (Ghiselli, Nardini, Baldi, & Scaccini, 1998; Simonetti, Pietta, & Testolin, 1997), catechins (Landraut, Poucheret, Ravel, Gasc, Cros, & Teissedre, 2001), proanthocyanidins (Rigo et al., 2000), anthocyanins (Burns, Mullen, Landrault, Teissedre, Lean, & Crozier, 2002; Ghiselli et al., 1998; Landraut et al., 2001), phenolic acids (Burns, Gardner, Matthews, Duthie, Lean, & Crozier, 2001) and stilbenes (Baderschneider & Winterhalter, 2000; Bums, Yokota, Ashihara, Lean, & Crosier, 2002; Goldberg, Ng, Karumanchiri, Diamandis, & Soleas, 1996; Ribeiro de Lima et al., 1999), but still the compounds inducing the possible health effects of wine have not yet been indicated. Lignans are a group of phenolic compounds, which occur in roots, leaves, seeds, fruits and wooden parts of vascular plants (Ayres & Loike, 1990). Two most extensively studied dietary lignans are secoisolariciresinol (Seco) and matairesinol (Mat) (Fig. 1). These two plant lignans have been analysed in fruits, vegetables, cereal products, tea and coffee (Liggins, Grimwood, & Bingham, 2000; Mazur, 1998; Mazur, Wähälä, Rasku, Salakka, Hase, & Adlercreutz, 1998; Nesbitt & Thompson, 1997). Secoisolariciresinol, lariciresinol and isolariciresinol with different conjugations have been identified in Riesling white wine (Baderschneider & Winterhalter, 2001). Secoisolariciresinol and matairesinol have also been quantified in a few different red wines (Mazur, 1998; Mazur & Adlercreutz, 2000).

Plant lignans, secoisolariciresinol and matairesinol, are metabolised in human to mammalian lignans enterolactone and enterodiol (Borriello, Setchell, Axelson, & Lawson, 1985). Because the urinary excretion of mammalian lignans exceeds the intake of secoisolariciresinol and matairesinol 10–12-fold, there must be some other mammalian lignan precursors in diet. Recently lariciresinol, pinoresinol, syringaresinol and isolariciresinol

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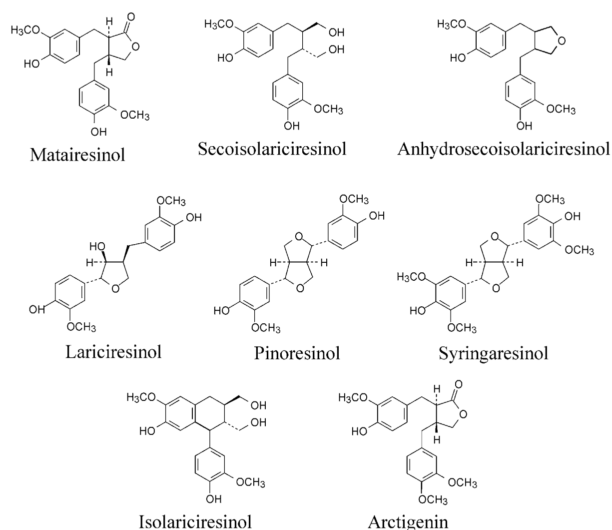

Fig. 1. Structures of the lignans.

were preliminary identified and quantified from rye bran. Lariciresinol and pinoresinol were also shown to act as precursors of mammalian lignans enterolactone and enterodiol (Heinonen, Nurmi, Liukkonen, Poutanen, Nishibe, & Adlercreutz, 2001), but the mammalian lignans were only minor metabolites of syringaresinol and arctigenin and isolariciresinol was not converted to enterodiol and enterolactone at all.

Mechanisms of actions of different phenolic compounds in humans are not known, but one very popular explanation is the antioxidant activity of compounds. Antioxidant activity of the plant lignans was studied after isolating the compounds from Riesling wine (Baderschneider & Winterhalter, 2001). Isolariciresinol had similar activity to that of quercetin-3-*O*-glucuronide and quercetin-3-*O*-rhamnoside when the activity of quercetin itself was 50% higher. Antioxidant activity of secoisolariciresinol diglucoside was studied in different models together with mammalian lignans enterolactone and enterodiol (Kitts, Yuan, Wijewickreme, & Thompson, 1999). It was shown that Seco diglucoside had lower activity in several tests compared with mammalian lignans enterolactone and enterodiol.

Phyto-oestrogens are known to affect on the production of sex hormone binding globulin, biological activity of the endogenous hormones and they inhibit the growth of tumour cells (Adlercreutz, 1995, 1998; Thompson, 1998). High serum enterolactone values have been associated with decreased risk of acute coronary event (Vanharanta, Voutilainen, Lakka, van der Lee, Adlercreutz, & Salonen, 1999) and decreased risk of breast cancer (Pietinen, Stumpf, Männistö, Kataja, Uusitupa, & Adlercreutz, 2001). Also association between low serum enterolactone and high plasma F_2–isoprostanes was recently shown (Vanharanta et al., 2002). F_2–isoprostanes indicate in vivo lipid peroxidation (Roberts & Morrow, 2000; Salonen, 2000). High urinary excretion of enterolactone has been associated with decreased risk of breast cancer (Adlercreutz, 1995; Ingram, Sanders, Kolybaba, & Lopez, 1997) even though also opposite finding has been reported (den Tonkelaar et al., 2001).

The aim of this study was to analyse the wide variety of different plant lignans in selected red and white wines consumed in Finland. We included into our study the known dietary lignans secoisolariciresinol and matairesinol, but also new mammalian lignan precursors lariciresinol and pinoresinol, which have not been earlier quantitatively analysed in wines. Furthermore, we wanted to study the amount of four other lignans syringaresinol, arctigenin, isolariciresinol and trachelogenin in wines.

2. Materials and methods

2.1. Standards and reagents

Pinoresinol (Pin) and syringaresinol (Syr) were isolated as described by Nishibe, Kinoshita, Takeda, and Okano (1990). Arctigenin was also isolated from plant material using the procedure of Chiba and co-workers (Chiba, Hisada, & Nishibe, 1978). Isolariciresinol (IsoL) (Makelä, Wähälä, & Hase, 2000), secoisolariciresinol (Seco), anhydrosecoisolariciresinol (Anse) and matairesinol (Mat), (Adlercreutz et al., 1986) were synthesized in the Laboratory of Organic Chemistry, University of Helsinki, Finland. Anhydrosecoisolariciresinol (Anse) is formed under acidic conditions from secoisolariciresinol diglucoside (Bakke & Klosterman, 1956). Glucose units are cleaved from the molecule and aliphatic hydroxyl groups react with acid resulting in an ester ring and a water molecule. To obtain the total amount of Seco in samples the measured molar amount of Anse is added to the measured amount of Seco. Dr. S. Ozawa and Dr. S. Nishibe (Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Japan) kindly provided lariciresinol (Lar) and trachelogenin (Tra). Structures of the lignans are presented in Fig. 1.

Methanol (MeOH) and acetonitrile (ACN) were HPLC-grade and purchased from Rathburn Chemicals Ltd. (Walkerbum, Scotland, UK). *Helix pomatia* enzyme mixture was purchased from Biosepra IBF/Sepracor (France) and diethylether from Merck (Darmstadt, Germany). QAE-Sephadex ion exchange material was obtained from Amersham Pharmacia Biotech AB (Uppsala, Sweden) and used in acetate form.

2.2. Chromatographic conditions

Samples were analysed using HPLC with coulometric electrode array detection (CEAD) (ESA Inc. Chelmsford,

MA, USA). HPLC system consisted of two pumps, autosampler with cooled sample tray and electrode array detector with eight electrode pairs. Detector cells and columns were kept at 37 °C in a thermostatically operated chamber. Mobile phase consisted of two eluents A: 50 mM sodium acetate buffer pH 5:MeOH (80:20 v/v) and B: 50 mM sodium acetate buffer pH 5:MeOH:ACN (40:40:20 v/v/v), which were earlier applied in plasma lignan analyses (Nurmi & Adlercreutz, 1999) and in the study of the in vitro metabolism of plant lignans (Heinonen et al., 2001). Flow rate was 0.3 ml/min and gradient elution was used. Gradient profile of the lignan method is presented in Table 1. Injection volume was 10 µl. Analytical column was Inertsil ODS-3 (GL Sciences Inc., Japan). Column dimensions were 150×3 mm and particle size of the end caped packing material was 3 µm. The guard column was a Quick Release C18 (Upchurch Scientific Inc., WA) with dimensions of 10×3 mm and packing material of 5 µm particles. Detection potentials were optimised according the hydrodynamic voltammograms of the analytes. Performance of the detector was evaluated by determining the limits of detection and linear ranges of each analyte. Resolution values were calculated with the following formula: $R = 1/4(\alpha - 1)\sqrt{N}[k/(k+1)]$ where N is plate number, α is separation factor and k is retention factor (Snyder, Kirkland, & Glajch, 1997). A standard chromatogram is presented in Fig. 2 to illustrate the resolution of the analytes.

Table 1
Gradient profile of the lignan method

	Lignans												
t/min	0	1.1	15	20	25	30	40	50	55	60	67	69	87
% B[a]	20	20	30	35	40	40	*55*	70	80	100	100	20	20

[a] Percentage amount of eluent B in the total flow. Composition of the eluent B is described in Section 2.

2.3. Samples

Detailed information of the samples is presented in Table 2 where grape variety, production region, country of origin and type of the wine is listed. Wines were chosen according to the list of the most consumed products in Finland 1999 except Spanish red wine number 3. Wines are identified with numbers and eight of the samples are red wines and two white wines. Three wines were produced in Spain, in Italy and in France and one originated from Hungary.

2.4. Sample pretreatment

Samples were pretreated according to the previously published method for food lignans (Mazur, Fotsis, Wähälä, Ojala, Salakka, & Adlercreutz, 1996). Alcohol was evaporated from the wine sample of 5 ml under N_2 flow keeping vials in water bath at 50 °C. Then wine was loaded into conditioned (6 ml of MeOH, 10 ml of water) C18 Sep-Pak cartridge. Sample was washed with 1.0 ml of water and eluted with 3.0 ml of MeOH. MeOH was evaporated under N_2 flow and the residue was dissolved in 0.5 ml of water. Hydrolysis reagent was 0.6 M sodium acetate buffer pH 4.1 containing 5000 U/ml β-glucuronidase from *Helix pomatia* and 5 mg/ml of ascorbic acid. 0.5 ml of hydrolysis reagent was added

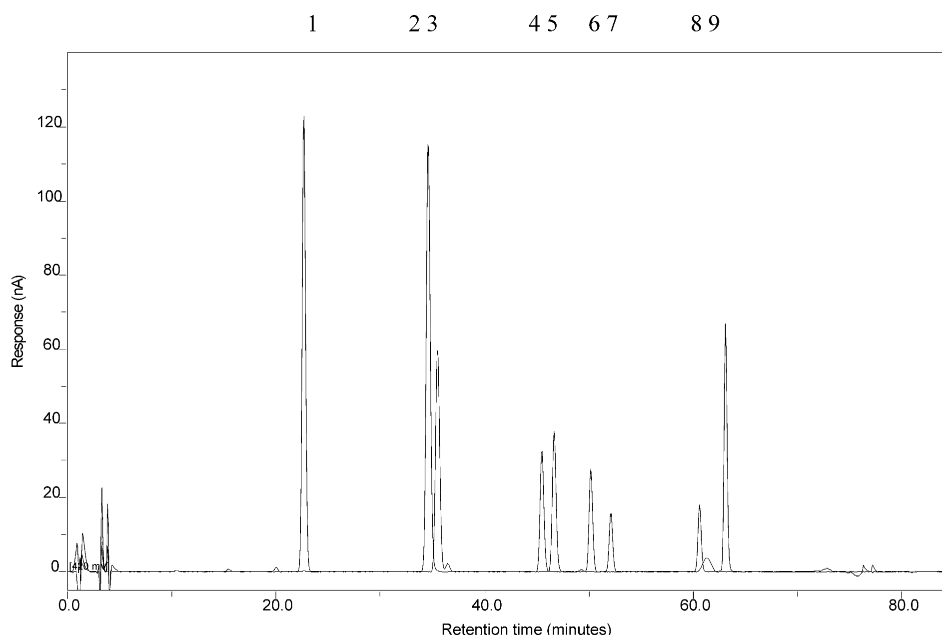

Fig. 2. Separation of the lignan standards. Numbers above correspond to the following compounds 1. Isolariciresinol, 2. Lariciresinol, 3. Secoisolariciresinol, 4. Syringaresinol, 5. Pinoresinol, 6. Matairesinol, 7. Trachelogenin, 8. Arctigenin and 9. Anhydrosecoisolariciresinol.

and sample was hydrolysed 2 h at 60 °C. After cooling sample was acidified with 10 µl of 6 M hydrochloric acid (HC1) and extracted twice with 5 ml of diethylether. Extracts were combined, evaporated under N_2 flow and dry residue was dissolved in 0.5 ml of MeOH. Water phase was further hydrolysed with 2 M HC1 (final concentration in the vial) 2.5 h at 100 °C. Cool sample was again extracted twice with diethylether, extracts were combined, evaporated and dry residue was dissolved in 0.5 ml of MeOH. Fractions from enzyme and acid hydrolysis were combined and the sample was further purified with QAE-Sephadex ion exchange chromatography in acetate form. Lignans were eluted with 4 ml of MeOH. Eluate was evaporated and the residue was dissolved in 1.0 ml of MeOH. This sample was then diluted with mobile phase containing 20% eluent B before the HPLC run.

3. Results

Detection potentials were chosen using the hydrodynamic voltammograms of the analytes. Detection potential gave the highest possible signal with sufficient signals on adjacent channels, too. Detection limits were determined with pure compounds and values with signal to noise ratio of 3 (S/N = 3) were accepted. Quantification limits were five times the values of detection limits. Linear range for each compound was determined by calculating the values using the least square method. Deviations of ±5% for the measured values from the calculated values were accepted. Detection limits of the lignans ranged from 2.4 (IsoL) to 12 (Anse and Mat) pg on column corresponding to 0.24 and 1.2 ng/ml, respectively. Linear ranges were up to 12 000 (IsoL, Mat, Anse) pg on column and correlation factors for linear ranges were from 0.9996 to 1.000. Upper limit of the linear range was not achieved in determination when ±5% error was used as a limiting value for the measured values. Detailed data is presented in Table 3. Resolution values for lignans in Table 3 indicated that Lar–Seco was a critical peak pair. The resolution for Lar–Seco was below 1.5, which is the limiting value of baseline separation for adjacent peaks of similar size (Snyder et al., 1997). Lignans had very narrow peaks with no tailing and peak height was used in quantification. Therefore Lar–Seco was possible to measure reliably.

Table 2
Selected wine samples

Sample[a]	Grapes	Region	Country	Sort
1	Tempranillo	Valencia	Spain	Red wine
2	Tempranillo	Valdepeñas	Spain	Red wine
3	Tempranillo	Valdepeñas	Spain	Red wine
4	Cabernet Sauvignon	Vin de Pays d'Oc	France	Red wine
5	Grenache, Cinsault, Carignan	Cote d'Or	France	Red wine
6	Sangiovese, Montepulciano, Uva di Troia	Apulia	Italy	Red wine
7	Lambrusco	Emilia-Romagnan	Italy	Red wine
8	Merlot	Eger	Hungary	Red wine
9	Garganega[b]	Veneto	Italy	White wine
10	Chasan	Vin de Pays d'Oc	France	White wine

[a] These numbers are used to identify the samples in Table 4 and in results.
[b] Seventy per cent of the grapes is Garganega and the rest is Chardonnay, Pinot Bianco and/or Trebbiano di Soave.

Table 3
Chromatographic parameters of the lignan method

Analyte	Retention time/min	Detection potential/mV	Detection limit/pg on column	Linear range/pg on column	Correlation factor for linear range	Resolution[a]
Isolariciresinol	23.43	420	2.4	12 000	1.0000	21.0
Lariciresinol	35.90	420	4.5	11 000	0.9998	1.2
Secoisolariciresinol	36.78	420	5.4	5400	1.0000	14.0
Syringaresinol	46.88	420	4.3	11 000	1.0000	1.8
Pinoresinol	48.06	420	4.5	4500	0.9996	5.4
Matairesinol	51.53	420	12	12 000	1.0000	3.2
Trachelogenin	53.53	420	10	10 000	1.0000	14.3
Arctigenin	62.04	420	11	2300	0.9999	4.5
Anhydrosecoisolariciresinol	64.46	420	12	12 000	1.0000	

[a] Resolution values were calculated with the following formula: $R = 1/4(\alpha - 1)\sqrt{N}[k/(k+1)]$. See details in text.

All wines had similar lignan profiles and an example of the chromatogram is presented in Fig. 3. The most abundant lignan in all wines was isolariciresinol representing on an average 63% (range from 56 to 71%) of the total lignans. The concentrations of the total lignans in red wines ranged from 0.812 (sample 5) to 1.406 (sample 3) mg/l. The amount of secoisolariciresinol in red wines ranged from 0.178 (sample 5) to 0.429 (sample 4) mg/l and the amount of matairesinol ranged from 0.028 (sample 4) to 0.067 (sample 1) mg/l. The mean concentrations of lariciresinol and syringaresinol were in red wines 0.054 and 0.038 mg/l. Arctigenin or trachelogenin was not detected in any wine and pinoresinol was not possible to quantify, because of co-eluting compound, which covered the peak of Pin. GC–MS identifications confirmed the presence of Pin on similar levels to Lar and Syr. The lowest amounts of all lignans were quantified in two white wines. Detailed results for individual lignans are presented in Table 4.

The amount of the mammalian lignan precursors in different red wines varied from 34 to 43% of the total lignans. The lowest amount of precursors in mg/l was detected in sample 5, which contained also the lowest amount of total lignans. The lowest percentage amount of precursors was detected in sample 3 containing the highest amount of total lignans. The percentage amount of the mammalian lignan precursors in the white wines was 10% lower than the average for the red wines. The amounts of the precursors are presented in Fig. 4.

4. Discussion

In this study, a wide variety of plant lignans in selected red and white wines were analysed. The only previous quantitative results for wine lignans were reported in two papers of Mazur where the total amount of wine Seco and Mat for one red wine was 1.38 mg/l (Mazur &

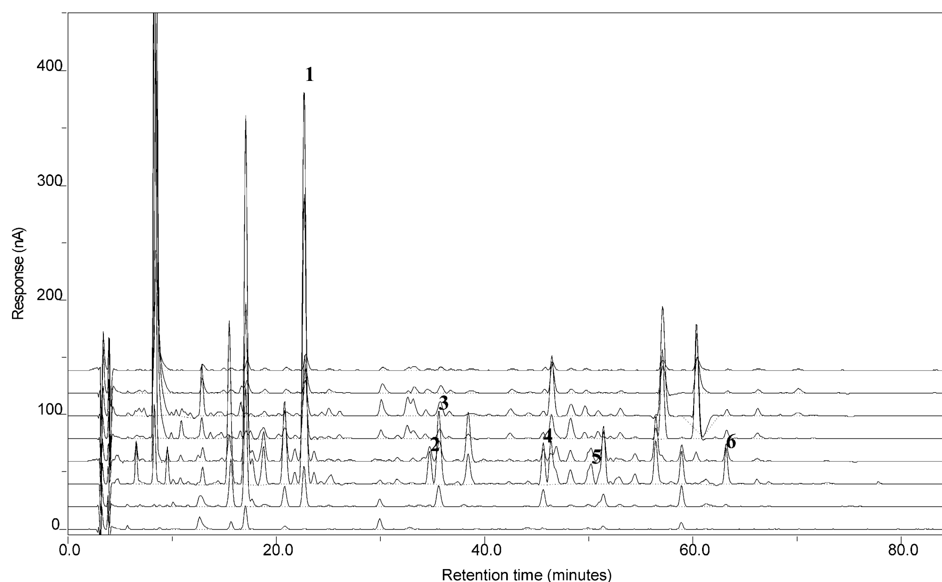

Fig. 3. Typical lignan chromatogram of the red wine. 1. Isolariciresinol, 2. Lariciresinol, 3. Secoisolariciresinol, 4. Syringaresinol, 5. Matairesinol, 6. Anhydrosecoisolariciresinol.

Table 4
Lignans in selected wines (mg/l)

Sample	Seco[a]	Mat	Lar	Syr	IsoL	Total lignans
1	0.318	0.067	0.071	0.015	0.752	1.223
2	0.228	0.038	0.050	0.033	0.587	0.936
3	0.257	0.051	0.066	0.110	0.922	1.406
4	0.429	0.028	0.024	0.018	0.662	1.161
5	0.178	0.028	0.042	0.020	0.544	0.812
6	0.292	0.038	0.071	0.024	0.796	1.221
7	0.375	0.039	0.062	0.026	0.731	1.233
8	0.248	0.029	0.041	0.038	0.626	0.982
9	0.038	0.010	0.035	0.010	0.261	0.354
10	0.097	0.021	0.040	0.019	0.464	0.641

[a] Abbreviations of the lignans are listed in Section 2.

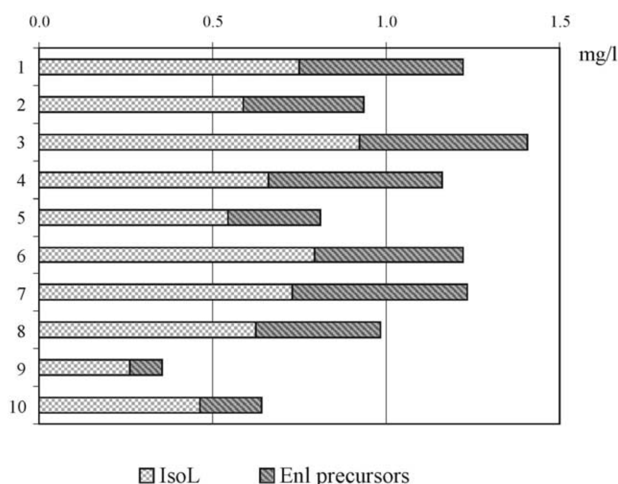

Fig. 4. The amount of enterolactone precursors in selected red and white wines. Sum of isolariciresinol and enterolactone precursors corresponds to the total lignans presented in Table 4.

Adlercreutz, 2000) and the range of the sum of these two lignans in four different red wines was 0.153–1.38 mg/l (Mazur, 1998). The lignan concentrations in our samples ranged from 0.354 to 1.406 mg/l and if only the red wines are included values ranged from 0.812 to 1.406 mg/l. The amount of lignans like the amount of other phenolic compounds in wines depends partly on the growing facilities and variety of the grapes used in vine making (Landraut et al., 2001; Soleas, Dam, Carey, & Goldberg, 1997). Therefore it was surprising that the variation in the concentrations were relatively small. In our study larger number of lignans was measured, but it was found out that only the amount of isolarciresinol in addition to Seco and Mat was really affecting on the total amount of lignans. Isolariciresinol was clearly a major plant lignan in all studied wines and the amount of the mammalian lignan precursors was not increased when compared with the previous results (Mazur, 1998; Mazur & Adlercreutz, 2000). On the contrary our results for Seco and Mat were slightly lower than those reported earlier (Mazur, 1998; Mazur & Adlercreutz, 2000). This may be due to differences in winemaking processes. Wine is stored in wood barrels, which may release some lignans into the products, and the amount of those lignans may depend on the storage time and conditions.

Plant lignan isolariciresinol possesses an equal antioxidant activity to different quercetin conjugates (Baderschneider & Winterhalter, 2001) and secoisolariciresinol diglucoside is less active than enterodiol and enterolactone (Kitts et al., 1999). To achieve the maximum benefit of the antioxidant activity of the wine lignans it is favourable that isolariciresinol is not converted to mammalian lignans and that secoisolariciresinol is metabolised to a great extent. Still in the red wines concentrations of lignans were 10–100 times lower compared with the concentration of flavonoids and phenolic acids (Landraut et al., 2001; Simonetti et al., 1997). Therefore the role of lignans in antioxidant activity of wines may be insignificant.

Regular consumption of red wine containing plant lignans may increase the mammalian lignan concentrations in serum to a level normally associated with decreased risk of diseases. However, increased serum enterolactone concentration has been also associated with intake of alcohol in general (Homer, Kristal, Prunty, Skor, Potter, & Lampe, 2002) and in one epidemiological study of breast cancer has been observed that the highest serum enterolactone concentrations do not necessarily always indicate decreased risk of disease (Hulten, Winkvist, Lenner, Johansson, Adlercreutz, & Hallmans, 2002). Similar amounts of lignans than from one liter of red wine can be obtained from 100 g of different nuts, berries and rye bread (Mazur, 1998; Mazur et al., 1996) together with fibre and other healthy components and without alcohol.

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References

Adlercreutz, H. (1995). Phytoestrogens: epidemiology and a possible role in cancer protection. *Environmental Health Perspectives*, *103*, 103–112.

Adlercreutz, H. (1998). Epidemiology of phytoestrogens. In H. Adlercreutz (Ed.), *Baillière's Clinical Endocrinology and Metabolism: Phyto-oestrogens* (pp. 605–623). London: Baillière Tindall.

Adlercreutz, H., Musey, P. I., Fotsis, T., Bannwart, C., WähAlä, K., Makelä, T., Brunow, G., & Hase, T. (1986). Identification of lignans and phytoestrogens in urine of chimpanzees. *Clinica Chimica Acta*, *158*, 147–154.

Ayres, D. C., & Loike, J. D. (1990). *Lignans: chemical, biological and clinical properties*. Cambridge University Press.

Baderschneider, B., & Winterhalter, P. (2000). Isolation and characterization of novel stilbene derivatives from riesling wine. *Journal of Agricultural and Food Chemistry*, *48*, 2681–2686.

Baderschneider, B., & Winterhalter, P. (2001). Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from riesling wine and screening for antioxidant activity. *Journal of Agricultural and Food Chemistry*, *49*, 2788–2798.

Bakke, J. E., & Klosterman, H. J. (1956). A new diglucoside from flaxseed. *Proceedings of the North Dacota Academy of Science*, *10*, 18–22.

Borriello, S. P., Setchell, K. D. R., Axelson, M., & Lawson, A. M. (1985). Production and metabolism of lignans by the human faecal flora. *Journal of Applied Bacteriology*, *58*, 37–43.

Burns, J., Gardner, P. T., Matthews, D., Duthie, G. G., Lean, M. E. J., & Crozier, A. (2001). Extraction of phenolics and changes in antioxidant activity of red wines during vinification. *Journal of Agricultural and Food Chemistry*, *49*, 5797–5808.

Burns, J., Mullen, W., Landrault, N., Teissedre, P.-L., Lean, M. E. J., & Crozier, A. (2002). Variations in the profile and content of anthocyanins in wines made from Cabernet Sauvignon and hybrid grapes. *Journal of Agricultural and Food Chemistry*, *50*, 4096–4102.

Burns, J., Yokota, T., Ashihara, H., Lean, M. E. J., & Crosier, A. (2002). Plant foods and herbal sources of resveratrol. *Journal of Agricultural and Food Chemistry*, *50*, 3337–3340.

Chiba, M., Hisada, S., & Nishibe, S. (1978). Studies on the Chinese crude drug "Forsythia Fructus"(III) on the constituents of fruits of *Forsythia viridissima* and *F. suspense*. *Shoyakugaku Zasshi*, *32*, 194–197.

den Tonkelaar, I., Keinan-Boker, L., Van't Veer, P., Arts, C. J. M., Adlercreutz, H., Thijssen, J. H. H., & Peeters, P. H. M. (2001). Urinary phytoestrogens and postmenopausal breast cancer risk. *Cancer Epidemiology, Biomarkers and Prevention*, *10*, 223–228.

German, J. B. (1998). Nutritional studies of flavonoids in wine. In C. A. Rice-Evans, & L. Packer (Eds.), *Flavonoids in health and disease* (pp. 343–358). New York: Marcel Dekker Inc.

Ghiselli, A., Nardini, M., Baldi, A., & Scaccini, C. (1998). Antioxidant activity of different phenolic fractions separated from Italian red wine. *Journal of Agricultural and Food Chemistry*, *46*, 361–367.

Goldberg, D. M., Ng, E., Karumanchiri, A., Diamandis, E. P., & Soleas, G. J. (1996). Resveratrol glucosides are important components of commercial wines. *American Journal of Enology and Viticulture*, *47*, 415–420.

Heinonen, S., Nurmi, T., Liukkonen, K., Poutanen, K., Nishibe, S., & Adlercreutz, H. (2001). In vitro metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *Journal of Agricultural and Food Chemistry*, *49*, 3178–3186.

Horner, N. K., Kristal, A. R., Prunty, J., Skor, H. E., Potter, J. D., & Lampe, J. W. (2002). Dietary determinants of plasma enterolactone. *Cancer Epidemiology, Biomarkers and Prevention*, *11*, 121–126.

Hulten, K., Winkvist, A., Lenner, P., Johansson, R., Adlercreutz, H., & Hallmans, G. (2002). An incident case-referent study on plasma enterolactone and breast cancer. *Europian Journal of Nutrition*, *41*, 168–176.

Ingram, D., Sanders, K., Kolybaba, M., & Lopez, D. (1997). Case-control study of phytooestrogens and breast cancer. *The Lancet*, *350*, 990–994.

Kitts, D. D., Yuan, Y. V., Wijewickreme, A. N., & Thompson, L. U. (1999). Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. *Molecular and Cellular Biochemistry*, *202*, 91–100.

Landraut, N., Poucheret, P., Ravel, P., Gasc, F., Cros, G., & Teissedre, P.-L. (2001). Antioxidant capacities and phenolics levels of French wines from different varieties and vintages. *Journal of Agricultural and Food Chemistry*, *49*, 3341–3348.

Liggins, J., Grimwood, R., & Bingham, S. A. (2000). Extraction and quantification of lignan phytoestrogens in food and human samples. *Analytical Biochemistry*, *287*, 102–109.

Mäkelä, T. H., Wähälä, K. T., & Hase, T. A. (2000). Synthesis of enterolactone and enterodiol precursors as potential inhibitors of human estrogen synthetase (aromatase). *Steroids*, *65*, 437–441.

Mazur, W., & Adlercreutz, H. (2000). Overview of naturally occuring endocrine-active substances in the human diet in relation to human health. *Nutrition*, *16*, 654–687.

Mazur, W., Fotsis, T., Wähälä, K., Ojala, S., Salakka, A., & Adlercreutz, H. (1996). Isotope dilution gas chromatographic–mass spectrometric method for the determination of isoflavonoids, coumesterol, and lignans in food samples. *Analytical Biochemistry*, *233*, 169–180.

Mazur, W., Wähälä, K., Rasku, S., Salakka, A., Hase, T., & Adlercreutz, H. (1998). Lignan and isoflavonoid concentrations in tea and coffee. *British Journal of Nutrition*, *79*, 37–45.

Mazur, W. M. (1998). Phytoestrogen content in foods. In H. Adlercreutz (Ed.), *Baillière's clinical endocrinology and metabolism: phyto-oestrogens* (pp. 729–742). London: Baillière Tindall.

Nesbitt, P. D., & Thompson, L. U. (1997). Lignans in homemade and commercial products containing flaxseed. *Nutrition and cancer*, *29*, 222–227.

Nishibe, S., Kinoshita, H., Takeda, H., & Okano, G. (1990). Phenolic compounds from stem bark of *Acanthopanax senticosus* and their pharmacological effect in chronic swimming stressed rats. *Chemical and Pharmaceutical Bulletin*, *38*, 1763–1765.

Nurmi, T., & Adlercreutz, H. (1999). Sensitive HPLC method for profiling phytoestrogens using coulometric array detection: application to plasma analysis. *Analytical Biochemistry*, *274*, 110–117.

Pietinen, P., Stumpf, K., Männistö, S., Kataja, V., Uusitupa, M., & Adlercreutz, H. (2001). Serum enterolactone and risk of breast cancer: a case-control study in eastern Finland. *Cancer Epidemiology, Biomarkers and Prevention*, *10*, 339–344.

Ribeiro de Lima, M., Waffo-Teguo, P., Teissedre, P. L., Pujolas, A., Vercauteren, J., Cabanis, J. C., & Merillon, J. M. (1999). Determination of stilbenes (*trans*-Astringin, *cis*- and *trans*-Piceid, and *cis*- and *trans*-Resveratrol) in Portuguese wines. *Journal of Agricultural and Food Chemistry*, *47*, 2666–2670.

Rigo, A., Vianello, F., Clementi, G., Rossetto, M., Scarpa, M., Vrhovsek, U., & Mattivi, F. (2000). Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some Italian red wines. *Journal of Agricultural and Food Chemistry*, *48*, 1996–2002.

Roberts, L. J., & Morrow, J. D. (2000). Measurement of F_2–isoprostanes as an index of oxidative stress in vivo. *Free Radical Biology and Medicine*, *28*, 505–513.

Salonen, J. T. (2000). Markers of oxidative damage and antioxidant protection: assessment of LDL oxidation. *Free Radical Research*, *33*, 41–46.

Simonetti, P., Pietta, P., & Testolin, G. (1997). Polyphenol content and total antioxidant potential of selected Italian wines. *Journal of Agricultural and Food Chemistry*, *45*, 1152–1155.

Snyder, L. S., Kirkland, J. J., & Glajch, J. L. (1997). *Practical HPLC method development* (2nd ed.). UK: John Wiley & Sons.

Soleas, G. J., Dam, J., Carey, M., & Goldberg, D. M. (1997). Toward, the fingerprinting of wines: cultivar-related patterns of polyphenolic constituents in ontario wines. *Journal of Agricultural and Food Chemistry*, *45*, 3871–3880.

Thompson, L. U. (1998). Experimental studies on lignans and cancer. In H. Adlercreutz (Ed.), *Baillière's clinical endocrinology and metabolism: phyto-oestrogens* (pp. 691–706). London: Baillière Tindall.

Vanharanta, M., Voutilainen, S., Lakka, T. A., van der Lee, M., Adlercreutz, H., & Salonen, J. T. (1999). Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study. *The Lancet*, *354*, 2112–2115.

Vanharanta, M., Voutilainen, S., Nurmi, T., Kaikkonen, J., Roberts, L. J., Morrow, J. D., Adlercreutz, H., & Salonen, J. T. (2002). Association between low serum enterolactone and increased plasma F_2–isoprostanes, a measure of lipid peroxidation. *Atherosclerosis*, *160*, 465–469.