

CHARACTERIZATION OF LIGNANS CONTENT IN DURUM WHEAT AND OTHER CEREALS

C. Platani^{1*}, R. Beleggia¹, A.M. Digesù¹, C. Fares¹, S. Moscaritolo², M.G. D'Egidio², L. Cattivelli¹

¹ CRA-Cereal Research Centre of Foggia, Italy (*e-mail address: cristiano.platani@entecra.it);

² CRA-Cereal Quality Research Unit of Rome, Italy



Introduction

The Lignans are one of major class phytoestrogenic compounds. They are present in a wide range of oilseed plants, vegetables and fruits with a number of biological effects, including decreased risk for hormone-dependant breast cancers, colon and prostate cancer and coronary heart disease (Vanharanta *et al.* 1999).

Flaxseed is among the richest known source of lignans, but the consumption of flaxseed products in human diets is relatively low. As a consequence, it is the cereal and whole-grain products, particularly rye and barley that provide the most important dietary source of lignans precursors. Lignan is a dimeric natural product derived by the combination of two phenylpropanoid C6-C3 units at β carbon atoms (Raffaelli *et al.* 2002). They are generally glycosidically linked to carbohydrates and in the large intestine are deconjugated from the carbohydrate portion by bacteria. Among the lignans present in cereal kernels, secoisolariciresinol and matairesinol are the best known precursors of mammalian lignans (enterodiol and enterolactone), nevertheless others components such as pinoresinol and lariciresinol, have also been recently identified as precursors of mammalian lignans (Heinonen *et al.* 2001).

In the last two decades, many analytical methods for detection and quantification of phytoestrogens and their metabolites in plant, plant derived products and biological matrices have been reported. GC-MS had been the most commonly used technique for the analysis of phytoestrogenic compounds and their metabolites in biological fluid due to its marked potential of high resolution, selectivity and sensitivity (Liggins *et al.* 2000). This work reports on the lignan content and on its components (isolariciresinol, matairesinol, lariciresinol, pinoresinol, secoisolariciresinol and anhydrosecoisolariciresinol) in different whole-kernel cereals through acidic hydrolysis of samples and successively GC/MS analysis.

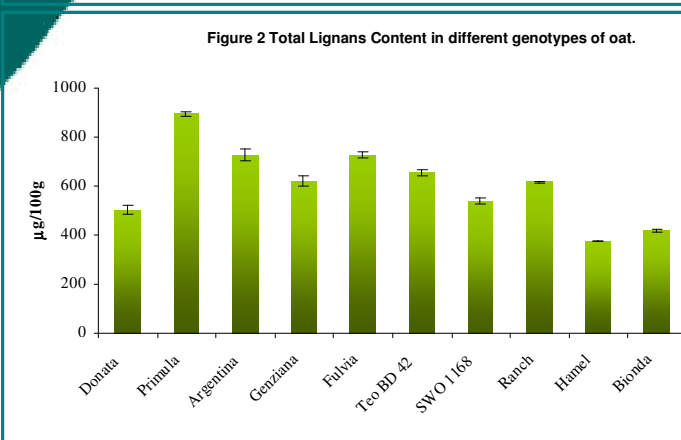
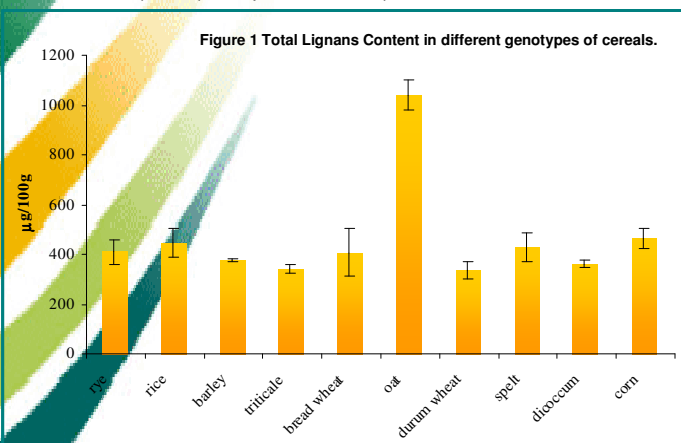
Materials and Methods

In this study a collection of various genotypes of cereals was employed. 1 g of defatted whole flour was hydrolysed with 5 mL of HCl 1.5M at 100°C for 150 minutes. The acidic solution was successively neutralized with NaOH 10M and extracted with Ethyl Acetate: Methyl-*tert*-butyl-ether (1:1 v/v). The organic layer was collected, 1 mL was dried under nitrogen stream and 1 mL of derivatization solution was added. The derivatization solution was composed from Pyridine, N,O-bis(trimethylsilyl)trifluoroacetamide (4:1 v/v) and trimethylchlorosilane (1%). As internal standard was employed Antraflavic Acid (2 μ g).

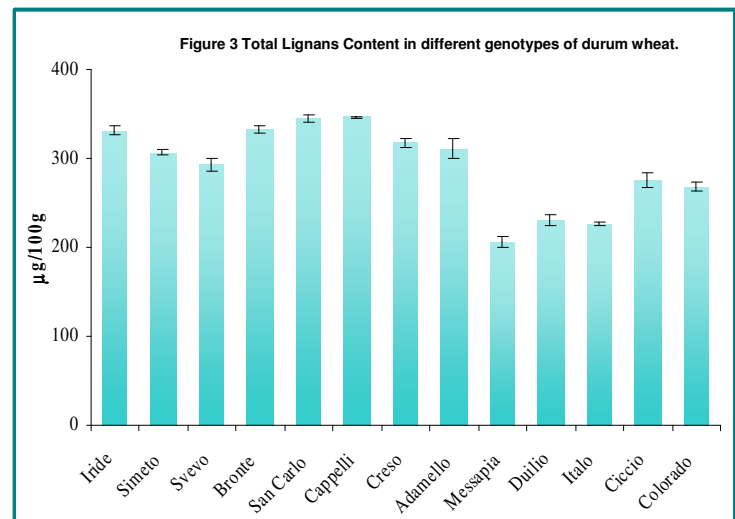
The derivatized sample was heated for 1h at 60°C and cooled at room temperature. Sample volume of 1 μ L was then injected into GC column in splitless mode connected to an Agilent 6890N gas chromatograph coupled with an Agilent 5973 quadrupole mass spectrometer. Gas chromatography was performed on a EQUITY 1 Supelco column (15m x 0,25mm i.d., 1 μ m). Injection temperature= 280°C, the transfer line T= 250°C and the ion source T= 230°C. Carrier gas: Helium at constant flow of 1 ml/min. The oven temperature program was: 180°C for 10 min, increase to 320°C (10°C/min), then the temperature was hold for 5 min. The acquisition spectra was in SIM. The identification of lignans was performed comparing the retention time with those of standards and the quantifications were obtained with the calibration curve response ratios of various concentrations of standards normalized with the internal standard.

Results and Discussion

In Figure 1 is reported the total lignans content of a genotype representation of different cereals (rye, rice, barley, triticale, bread wheat, oat, durum wheat, spelt, dicoccum and corn). Among the cereal species investigated, the variability range from 336,1 to 1040,3 μ g/100g and, as showed from the graphic, the highest and the lowest content of total lignans were observed in oat and durum wheat samples respectively while the other species showed a similar values.



On the basis of these data, a range of cultivars representing a sample of the genetic variability of oat and durum wheat was analysed and the results are showed in Figures 2 and 3. In oats, the variability across cultivars ranged from 895,1 μ g/100g (cv Primula) to 375,9 μ g/100g (cv Hamel), while in durum wheat the range of variability for lignan content was between 346,3 μ g/100g of the cv Cappelli and 205,8 μ g/100g of the cv Messapia.



The cultivars, beside a different lignan content, also showed a different lignan composition. In Table 1 is reported the percentage composition of the two cultivars from oat and durum wheat genotypes showing the highest and the lowest content of total lignans. Primula and Cappelli showed the great content of isolariciresinol (43% and 40% respectively) while Hamel and Messapia were characterized from the highest percentage of secoisolariciresinol and anhydrosecoisolariciresinol (47% and 35% respectively). The higher values of matairesinol and lariciresinol were observed in Cappelli and Primula respectively. In general, pinoresinol was the minor component in all cultivars.

Table 1 Percentage composition in oat and durum wheat cultivars

	Isolariciresinol	Matairesinol	Pinoresinol	Lariciresinol	Secoisolariciresinol + Anhydrosecoisolariciresinol
Primula (oat)	43%	3%	1%	35%	18%
Hamel (oat)	19%	9%	8%	17%	47%
Cappelli (durum wheat)	40%	16%	9%	10%	25%
Messapia (durum wheat)	31%	13%	7%	14%	35%

Conclusion

The results obtained in this preliminary study showed a wide variability among the cereal species investigated for the total lignan content and, between the oat and durum wheat genotypes, it was observed also a significant difference of the individual lignan components.

References

- Vanharanta, M.; Voutilainen, S.; Lakka, T.A.; van der Lee, M.; Adlercreutz, H.; Salonen, J.T. Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study. *Lancet* **1999**, *354*, 2112-2115
- Raffaelli, B.; Hoikkala, A.; Leppälä, E.; Wähälä, K. Enterolignans. *J. Chrom. B* **2002**, *777*, 29-43
- Heinonen, S.; Nurmi, T.; Liukkonen, K.; Poutaten, K.; Wähälä, K.; Deyama, T.; Nishibe, S.; Adlercreutz, H. In vitro metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *J. Agric. Food Chem.* **2001**, *49*, 3178-3186
- Liggins, J.; Grimwood, R.; Bingham, S.A. Extraction and quantification of lignan phytoestrogens in food and human samples. *Anal. Biochem.* **2000**, *287*, 102-109