In vitro Binding of Pyridoxal 5' - Phosphate and Tryptophan to Isolated Dietary Fibres from Rapeseed and Lupins

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INTRODUCTION

European grown oil and protein crops comprise mainly rapeseed and various legume seeds, including pea, faba bean and lupin, which is expected to gain more interest as agricultural crops in the future. The applicability of rapeseed and lupin seeds for feed and food purposes may however be hindered by the co-occurrence of dietary fibres (DF) and various antinutrients, which in different ways can reduce optimal utilisation of energy, protein as well as other major and minor seed constituents (Bjergegaard *et al.*, 1991; Danielsen *et al.*, 1994; Edwards & van Barneveld, 1998). DF in food and feed has also beneficial effects owing to their binding of xenobiotics or antinutritional food constituents (Bjergegaard *et al.*, 1998). Utilisation of beneficial effects and reduction of negative effects of DF require specific knowledge to structure-functionality relations of DF.

DF is traditionally defined as "non-starch polysaccharides and lignins", but actually this fraction, being mainly plant cell wall derived, is much more complex having a wide range of non-carbohydrate components associated (Cho *et al.*, 1997). Interest has, both in connection with beneficial and as mentioned above, antinutritional effects of DF, been focused on the physico-chemical properties of DF, including among others binding of water, bile acids, fat, vitamins, xenobiotica, and various other organic and inorganic compounds, e.g. minerals (Platt & Clydesdale, 1987; Story & Lord, 1987; Nnanna & O'Neill, 1992; Marques Mendes *et al.*, 1993; Ralet *et al.*, 1993).

The aim of the present study was to determine the binding capacity of isolated insoluble DF (IDF) and soluble DF (SDF) from rapeseed and lupin towards tryptophan (Trp) and vitamin B6 (pyridoxal-5'- phosphate; B6). The essential amino acid, tryptophan, may not be the first limiting, but the indolyle structure indicates that e.g. lignins or DF-associated phenolics may have a hydrophobic affinity towards this. This is also a valid argument for vitamin B6. The method used in the study now described involved transfer of isolated soaked DF to small dialysis bags placed in different test solutions followed by registration of UV-absorption after fixed time-intervals.

MATERIALS AND METHODS

IDF and SDF were isolated from *Brassica napus* L. (cv. Apex) and *Lupinus luteus* L. (cv. Juno) by an enzymatic gravimetric procedure (Asp *et al.*, 1983; Bjergegaard *et al.*, 1991). The starting material comprised rapeseed hulls and protein rich rapeseed meal (PRM), derived from aqueous enzymatic extraction of rapeseed oil, as well as lupin hulls and dehulled lupin meal.

About 200 mg IDF or SDF were weighed into dialysis bags (cut-off value: 8000 g/mol) and 4 ml water was added to hydrate the sample. Dialysis was performed for 24 hours to remove any UV absorbent compounds released from DF. The dialysis bags were then placed in the

test solutions (B6; 0.61 mM or Trp; 0.21 mM). After 15 minutes in the thermostatic controlled waterbath (37°C), 3 ml of the test solution was removed and used for determination of the absorption spectrum from 190-400 nm with specific determination at 387 nm (B6) or 280 nm (Trp). The 3 ml was then returned to the solution, and the described procedure was repeated with varying intervals up to 27 hours from starting time.

Three blanks were included in the study. The first blank was a dialysis bag containing IDF or SDF and 5 ml water placed in 30 ml water in a conical flask to control any UV absorbent compound released from IDF and SDF and from the dialysis bag. The second blank was a dialysis bag containing only 5 ml water placed in a conical flask containing 30 ml water to control any UV absorbent compound released from the dialysis bag alone. The third blank was a dialysis bag containing only 5 ml water placed in a conical flask containing 30 ml test solution to control the test solution dilution and possible breakdown of test-compounds over time. Any UV absorbent compound released from the dialysis bags is also included in the third blank.

RESULTS AND DISCUSSION

The binding capacity towards B6 of IDF and SDF, isolated from rapeseed hulls and PRM are summarized in Figure 1.

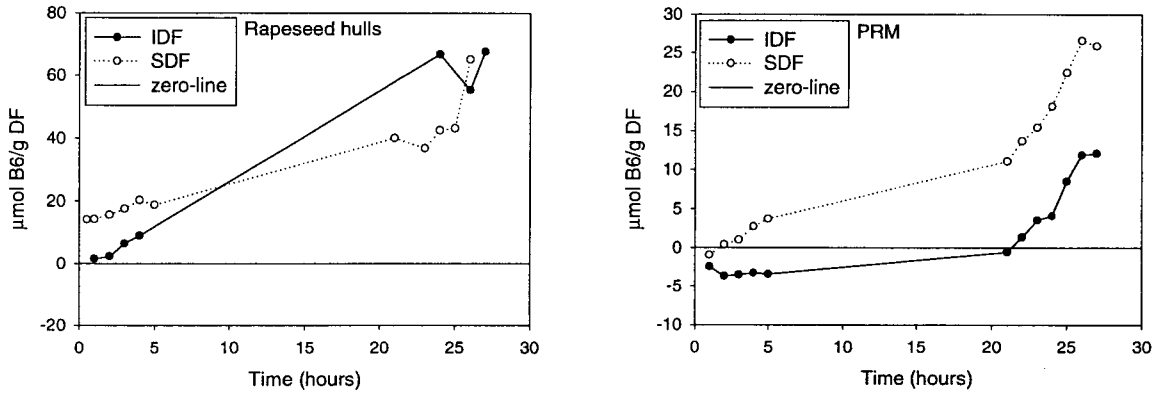

Figure 1. Binding capacity of DF from rapeseed hulls (left) and PRM (right) towards pyridoxal-5'- phosphate. The figures presented are based on the change in absorption over time (387 nm) and are corrected for blank values.

The results in Figure 1 shows, that DF from rapeseed hulls had a markedly stronger binding capacity towards B6 than DF from the dehulled rapeseed product PRM, and the affinity generally increased with time. Solubility based fractionation of the total DF fraction (TDF = SDF + IDF) into the subfractions "pectins", "hemicelluloses", "cellulose", and "lignin" showed as expected a high level of lignin in DF from rapeseed hulls (82%), whereas the corresponding level in PRM was less than 10%. The polyphenolic character of lignin, which will be present in the IDF fraction together with cellulose and some insoluble hemicelluloses, makes adsorption of B6 likely, and this may be one of the explanations for the good binding capacity of IDF from the hull fraction. The SDF fraction is dominated by pectins and some soluble hemicelluloses, polysaccharides which are not expected to have any affinity to B6.

Factors other than lignin content, which may be of interest discussing binding of B6 as well as Trp, comprise DF-associated proteins and DF-associated low-molecular weight (LMW)

phenolics as e.g. cinnamic acid derivatives. Table 1 shows the content of protein (N x 6.25), LMW phenolics and supercritical fluid (SF) extractable compounds in DF from sources tested in the binding trials.

Table 1. Content of protein (N x 6.25), LMW phenolics and SF extractable compounds in isolated DF from rapeseed hulls, PRM, lupine hulls, and dehulled lupine meal. SF-extraction was performed at 50 MPa and 75°C, and the extraction time was 30 min. (pure CO_2) followed by 60 min. (MeOH modified CO_2). More information about the SF extraction can be found in Andersen *et al.* (1998a). Figures in brackets are relative standard deviations (%).

Plant material	DF-type	Protein (% of DF)	LMW phenolics[1)2)] (nmol/g DF)	SF extractable material[1)] (mg/g DF)
Rapeseed hull	IDF	20,4 (0,6)		
	SDF	40,2 (1,1)		
	TDF		1272 (5)	126,0 (10,4)
PRM	IDF	38,7 (0.9)		
	SDF	59,5 (0,7)		
	TDF		2028 (17)	196,0 (6,7)
Lupine hull	IDF	6,6 (0,1)		
	SDF	47,0 (0,4)		
	TDF		261 (15)	19,8 (7,9)
Dehulled lupin meal	IDF	13,5 (0,3)		
	SDF	56,5 (0,1)		
	TDF		416 (7)	131,6 (9,7)

[1)]Data obtained from Andersen *et al.* (1998a)
[2)]LMW phenolics is quantified from UV spectroscopy of SF extractable material (MeOH modified CO_2) (325 nm; ε-value: 15000 $M^{-1}cm^{-1}$)

As seen from Table 1, the binding capacity of SDF towards B6 is may be explained by other DF-associated non-carbohydrate components than lignin. Former studies have revealed a high level of cinnamic acid derivatives associated to the DF fraction of protein rich meal from rapeseed (Andersen *et al.,* 1998b), and the level of DF SF extractable material, which is of hydrophobic character, is clearly higher in PRM compared to rapeseed hulls. Rapeseed contains about 4-8 times more IDF than SDF, and IDF will thus be expected to exert the greatest effect on B6 availability in the gastrointestinal tract.

SDF from lupin meal and PRM showed comparable binding capacities towards B6, whereas lupin hull SDF exhibited slightly lower binding than rapeseed hulls SDF. The level of DF associated phenolics in lupin meal are considerable lower than in PRM, and other compounds have to be responsible for the adsorption observed, e.g. DF-associated proteins. The binding capacity of IDF in lupin compared to rapeseed was negligible. The lignin content in TDF from lupin hulls was found to be around 40%, whereas the level in TDF from lupine meal was comparable to PRM. Moreover, both LMW phenolics and SF extractable material is present in considerably higher level in rapeseed hull DF than in DF isolated from lupin hulls (Table 1).

The binding capacity towards Trp of IDF isolated from rapeseed and lupine hulls are presented in Figure 2

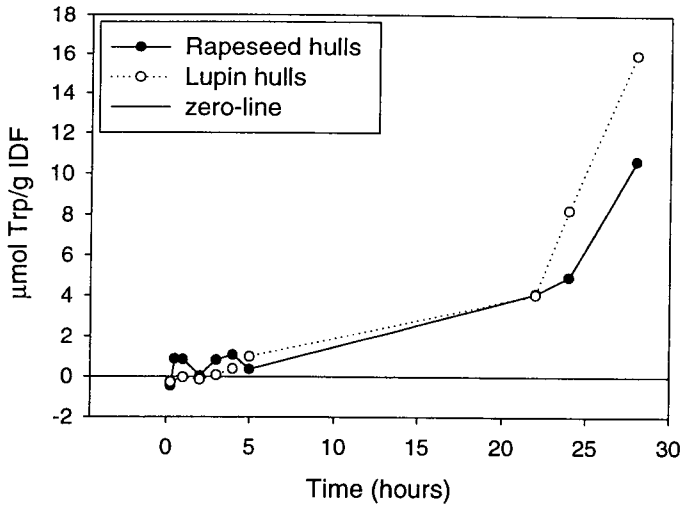

Figure 2. Binding capacity of IDF from rapeseed and lupin hulls towards tryptophan. The figures presented are based on the change in absorption over time (280 nm) and are corrected for blank values.

Data obtained from Figure 2 show a tendency to increased Trp binding capacity of IDF from lupin hulls compared to IDF from rapeseed hulls. The progress of binding was slow for the first 24 hours after when there was seen a marked increase in affinity for Trp. This was valid for both DF sources and is possibly a result of some unfolding effect, which was also observed in the binding trials for B6. As discussed above, the lignin content, which may be a possible candidate for binding compounds of indolyle structure, are approximately a factor 2 higher in rapeseed hull IDF compared to lupine hull IDF. Moreover, neither the level of DF associated LMW-phenolics, DF-associated protein nor the content of SF extractable material is indicative for the difference observed.

Comparison between B6 and Trp binding capacities of IDF showed that rapeseed hull fibres had a markedly higher affinity for B6 than for Trp. The structures of B6 and Trp are shown in Figure 3.

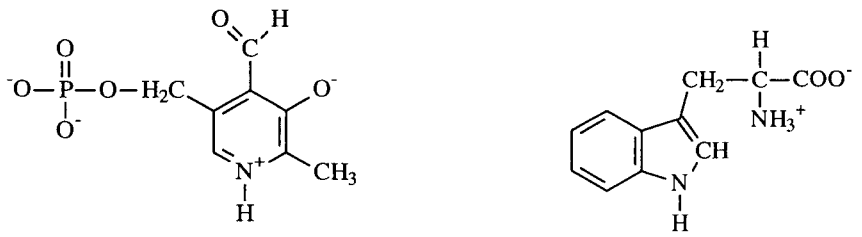

Figure 3. Structures for pyridoxal-5'-phosphate and tryptophan at neutral pH. The binding trials were performed in aqueous solution with a pH around 6-7.

An explanation for the difference in affinity for B6 and Trp may possibly be found in the hydrophilic part of the molecules, where B6, due to the phosphate group, more likely than tryptophan would participate in hydrogen bonds to the polysaccharide part or ion bonds to the proteins of the DF fraction. It should be stated that the results presented here were obtained in an *in vitro* system, which can not be correlated directly to *in vivo*. Firstly, the isolation procedure for DF may cause changes in organisation and chemistry of DF components, and

consequently alter the binding characteristics compared to DF present in the plant cell wall of an intact feed/food source. Another important issue is the much more complex system existing *in vivo* compared to the simplified *in vitro* conditions for the binding trials. The consumption of a mixed diet/feed introduces components, which, together with the general passage of the gastrointestinal tract, may change the ability of DF to bind vitamins and other LMW organic compounds.

CONCLUSION

The results indicate that DF from various rapeseed and lupin fractions bind LMW organic compounds as represented by B6 and Trp, and the affinity towards B6 is found to be clearly higher than the affinity towards Trp. The binding capacity of DF isolated from rapeseed hulls is the best of the DF sources tested. Various factors may influence binding capacities, and possible the non-carbohydrate part of the DF fraction is the most important in this respect. Components of interest is the polyphenolic lignin fraction, LMW phenolics, including various cinnamic acid derivatives, proteins, especially glycoproteins, and various other hydrophobic compounds.

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REFERENCES

Andersen, K.E., Bjergegaard, C., and Sørensen, H. (1998a). Characterisation of dietary fibre associated constituents based on supercritical fluid extraction. Pol. J. Food Nutr. Sci. 7/48(4):617-630.

Andersen, K.E., Bjergegaard, C., and Sørensen, H. (1998b). Dietary fibre associated compounds in different rapeseed products. GCIRC Bulletin, 15:90-95.

Asp, N. G., Johansson, C. G., Hallmer, H., and Siljeström, M. (1983). Rapid enzymatic assay of insoluble and soluble dietary fiber. J. Agric. Food. Chem. 31: 476-482.

Bjergegaard, C., Eggum, B. O., Jensen, S.K., and Sørensen, H. (1991). Dietary fibres in oilseed rape: Physiological and antinutritional effects in rats of isolated IDF and SDF added to a standard diet. J. Anim. Physiol. a. Anim. Nutr., 66: 69-79.

Bjergegaard, C, Jensen, S.K., Quinsac, A., and Sørensen, H. (1998). Analyses of antinutritional factors in rapeseed. In: Proceedings of the 3rd International Workshop on "Antinutritional factors in legume seeds and rapeseed", 8-10 July, Wageningen, The Netherlands, pp. 67-90.

Cho, S., Devries, J.W., and Prosky, L (1997). Dietary fiber analysis and applications. AOAC International, Maryland, USA, 202 pp.

Danielsen, V., Eggum, B.O., Jensen, S.K., and Sørensen, H. (1994). Dehulled protein-rich rapeseed meal as a protein source for early weaned piglets. Anim. Feed. Sci. Tech. 46:239-250.

Edwards, A.C. and van Barneveld, R.J. (1998). Lupins for livestock and fish. In: Lupins as Crop Plants, Biology, Production and Utilization (Eds. J.S. Gladstones, C.A. Atkins, and J. Hamblin), CAB International, Wallingford, UK, pp.385-405.

Marques Méndez, M.H., Nova Derivi, S.C., Fernandes, M.L., and Gomes de Oliveira, A.M. (1993). Insoluble dietary fiber of grain food legumes and protein digestibility. Archivos Latiniamericanos de Nutricion 43(1):6-72.

Nnanna I. A. and O`Neill K. L. (1992). In *vitro* binding of vitamin E to selected dietary fiber sources. J. Food Sci., 57 (3): 721-725.

Platt, S. R., and Clydesdale, F. M. (1987). Mineral binding characteristics of lignin, guar gum, cellulose, pectin and neutral detergent fiber under simulated duodenal conditions. J. Food Sci., 52:1414-1419.

Ralet, M.-C., Della Valle, G., and Thibault, J.-F. (1993). Raw and extruded fribre from pea hulls. Part I: Composition and physico-chemical properties. Carbohydrate Polymers 20:17-23.

Story, J.A. and Lord, S.L. (1987). Bile salts: In vitro studies with fibre components. Scand. J. gastroenterol., 22 (suppl. 129):174-180.