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Dietary Fibre Associated Compounds in Different Rapeseed Products

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INTRODUCTION

Dietary fibres (DF) comprise a heterogeneous group of mainly plant cell wall derived constituents resistant to hydrolysis by the alimentary enzymes of man. DF constitutes about 20-25% of the dry matter in double low rapeseed varieties with the main constituents being non-starch polysaccharides (NSP), proteins (*e.g.* glycoproteins) and lignins, whereas minor components comprise various non-carbohydrate compounds such as various lipids and phenolics (*e.g.* tannins, lignans, cinnamic acid derivatives) (Bjergegaard *et al.*, 1991; Bjergegaard *et al.*, 1994; Ochodzki *et al.*, 1995; Bjergegaard *et al.*, 1997). The constituents of the plant cell wall exist as a complex network of individual components closely associated to each other, either by non-covalent or covalent linkages. Although present in relatively low levels, even the minor DF associated constituents may thus have great impact on the physico-chemical properties and hereby the physiological effects of the DF fraction as a whole.

The aim of the present work has been concentrated on rapeseed DF associated compounds with focus on chemical characterisation of low molecular weight non-carbohydrate constituents including different cinnamic acid derivatives studied by UV and micellar electrokinetic capillary chromatography (MECC). For this purpose, DF was isolated by an enzymatic gravimetric procedure and DF associated components was then extracted by supercritical fluid extraction (SFE). SFE is a relatively new technique in analytical connection, in which an extraction media, often CO_2 possibly added a polar modifier (*e.g.* methanol), is used as extractant at elevated pressure and temperature depending on the critical parameters of the actual fluid mixture. SFE has proven effective for the extraction of lipophilic and amphiphilic compounds due to high diffusivity and low viscosity of supercritical fluids giving a higher analyte transport in matrix compared to liquid solvents (Buskov *et al.*, 1997a; Li *et al.*, 1997). The investigated plant material comprised seeds of double low winter rape (*Brássica napus* L. cv. 'Apex' (RS)) as well as various rapeseed products obtained from biorefining of RS by aqueous enzyme based processing resulting in rapeseed oil, protein rich meal (PRM), lipoprotein (LIPRO) and rapeseed hulls (RH). This processing was performed at Bioraf Denmark's pilot plant in Åkirkeby, Bornholm, Denmark (Jensen *et al.*, 1990; Ochodzki *et al.*, 1995; Bagger *et al.*, 1996).

RESULTS AND DISCUSSION

The effectiveness of the SFE procedure was investigated by a study of the extraction time required for lipid extraction using DF isolated from seeds of rape and lupine as starting material (1 g) (Andersen *et al.*, 1998 (in press)). Results from this study using pure CO_2 as extractant (75°C, 50 MPa) for 30 min showed almost complete extraction of the more lipophilic compounds including fat (oil) within the first 30 min regardless of the DF type used. After this first extraction, the polarity of the fluid was then changed by addition of MeOH (150 mL MeOH/L for 60 min followed by 250

mL MeOH/L for the next 60 min). This resulted in further extraction of material from both DF types, the RS DF releasing the extractable compounds more slowly than lupine DF.

Table 1 shows the gravimetric results for SFE of DF from the different rapeseed products investigated. Extraction was performed by pure CO_2 for the first 30 min (SFE A), and with added modifier (150 mL MeOH/L fluid in CO_2) for the next 30 min (SFE B) (75°C, 50 MPa).

Table 1. Gravimetric determination of DF associated compounds extracted by SFE. The results are given as mg extractable material/g DF.

DF from:	SFE A	SFE B	SFE Total
RS	105.6	33.9	136.6
LIPRO	41.8	27.3	69.4
PRM	152.6	49.6	196.0
RH	105.8	20.9	126.0

The highest yield was obtained by extraction with pure CO_2, and addition of modifier resulted in extraction of further 20-50% material. DF from PRM (a dehulled material) showed the highest level of extractable compounds (20% of weight of starting material), whereas extraction of DF from LIPRO only resulted in a total yield of about 7% of the starting material. Part of the compounds extracted to SFE A is possibly various fats. The extracts were investigated by spectroscopic analysis, measuring the absorbance at 278-280 nm as a measure for proteins, 325-330 nm as a measure for cinnamic acid derivatives, 470-474 nm as measure for carotenoides, and 667-669 nm as a measure for chlorophyll. The results are shown in Table 2.

Table 2. UV-Vis spectroscopy of DF associated compounds extracted by SFE. Quantification of extractable compounds was performed using Lambert Beer's law. Protein contents were quantified using $E^{1\%}_{1cm} = 10$ *whereas cinnamic acid derivatives, carotenoides, and chlorophyll were quantified using the ε-values: 15000, 2280 and 8,63 x* 10^{-4} *L* mol^{-1} cm^{-1}*, respectively.*

	DF from:							
	RS		LIPRO		PRM		RH	
	SFE A	SFE B	SFE A	SFE B	SFE A	SFE B	SFE A	SFE B
Protein (mg/g)	23	17	9	20	48	35	27	18
Cinnamic acid derivatives (nmol/g)	391	1193	169	1248	0	2028	855	1272
Carotenoides (nmol/g)	189	0	182	0	0	0	263	0
Chlorophyll (nmol/g)	1	5	2	6	1	1	2	2

The determinations (Table 2) showed a generally higher protein content in SFE A extracts compared to SFE B extracts except for DF from LIPRO. The types of proteins present in the extracts are yet to be determined but SFE B proteins are probably of more polar type than the SFE A proteins.

The level of cinnamic acid derivatives was clearly highest in the more polar SFE B extract, indicating the presence of various sinapoylderivatives as *e.g.* sinapoyl esters of choline, malate and carbohydrates. A problem in CO_2-based SFE is the presence of water during extraction as this may

lower pH of the fluid by formation of carbonic acid. This again may result in breakage of ester linkages by acid catalysed hydrolysis and thereby liberation of otherwise non-extractable covalently attached DF components. Moreover, protonisation of ionic components due to low pH may lead to extraction by the SFE A procedure using pure CO_2, as also indicated by the presence of cinnamic or sinapic acid derivatives in SFE A extracts.

Carotenoids were extracted to the SFE A extracts from RS, LIPRO, and RH DF, whereas these apolar compounds as expected were absent from the SFE B extracts. The SFE B extracts contain also amphiphilic compounds as *e.g.* phospholipids (Andersen *et al.*, 1997), but these compounds do not absorb UV light at 235 nm and they are not considered in the present work. The small amounts of chlorophylls detected in DF are thought to be adsorbed to the DF fraction during isolation, as chlorophylls in the intact plant are located in small subcellular organelles (chloroplasts).

It is noteworthy how the amount and type of DF associated non-carbohydrate compounds depend on the origin of the DF fraction. Marked differences have *e.g.* been demonstrated for DF from the hull fraction of rapeseed (RH) compared to DF from the dehulled product PRM (Tables 1 and 2), and the variations get even more pronounced when comparing DF from cereals and legumes with DF from oil seed rape (Andersen *et al.* 1997, Andersen *et al.*, 1998 (in press)). In order to get more close to the nature of the extractable compounds, micellar electrokinetic capillary electrophoresis (MECC) was performed for SFE A as well as SFE B extracts. Prior to analysis, the samples were prepared by dissolving the extract in 2 ml 1-PrOH, from which 200 µL then was evaporated, redissolved in 500 µL 1 mol/L NH_3 and re-evaporated to complete dryness. Finally, 100 µL H_2O was added prior to analysis. An example of an electropherogram of different groups of compounds, which are of interest for this work, is shown in Figure 1.

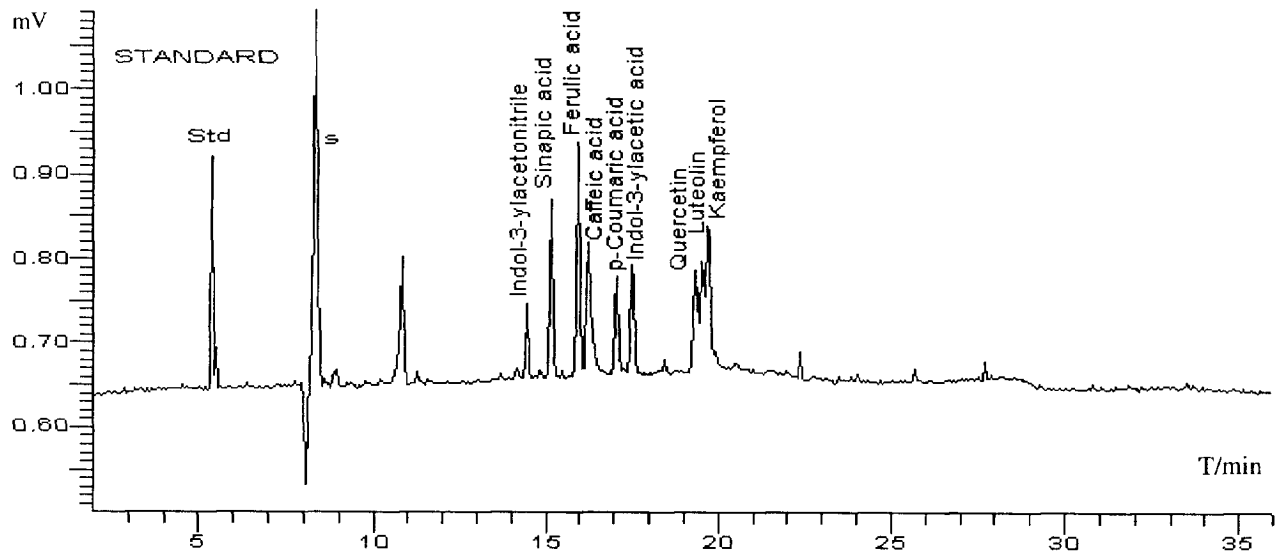

Figure 1. MECC of various phenolic acid derivatives, flavonoids, and other selected compounds. Analysis was performed using an ABI Model 270A-HT capillary electrophoresis system (Applied Biosystems, Foster City, CA, USA) with a 760 x 0.05 mm I.D. fused-silica capillary. Detection (235 nm) was performed on-column 530 mm from inlet. The separation was performed at a temperature and voltage of 30°C and +20 kV, respectively. The buffer system consisted of 400 mmol/L taurine, 15 mmol/L Na_2HPO_4, 140 mmol/L cholic acid and 2 % 1-PrOH (Buskov et al., 1997b). 0.1 mol/L trigonelline amide was used as external standard.

The separation principle in MECC is based on a distribution of analytes between the aqueous buffer phase and the so-called pseudostationary micellar phase in combination with differences in electrophoretic mobility of analytes. In the cholate system, the micelles are negatively charged and basically migrate towards the anode opposite to the electroosmotic flow, which pull the micelles against their charge towards the outlet at the cathode. Compounds having a high interaction with the micelles will be delayed relatively more than compounds with little or no interaction with this phase. As seen from Figure 1, the internal standard (Std; trigonelline amide) being positively charged migrate fast through the system, whereas the negatively charged phenolic acid derivatives (sinapic, ferulic, caffeic and coumaric acid) is placed in a group about 7-9 minutes after the solvent front (s). The aromatic choline ester sinapine, having a positive charge, is placed in close association with the solvent front, whereas tyrosine and tryptophan with zero netcharge (not shown) is placed just behind the solvent front. Two compounds of interest in connection with regulation of plant growth has also been included in this electropherogram, namely indol-3-ylacetonitrile and indol-3-ylacetic acid, which is placed just before and just after the group of free phenolic acids. It is informative to see how the negative charge on indol-3-ylacetic acid delay its migration compared to the neutral indol-3-ylacetonitrile. Finally, the flavonoid aglycones: quercetin, luteolin and kaempferol are examples of neutral compounds having high interaction with the micellar phase, compounds which as expected is found relatively late in the electropherogram.

Figure 2 shows an example of RS DF, SFE B analysed by the MECC system described above.

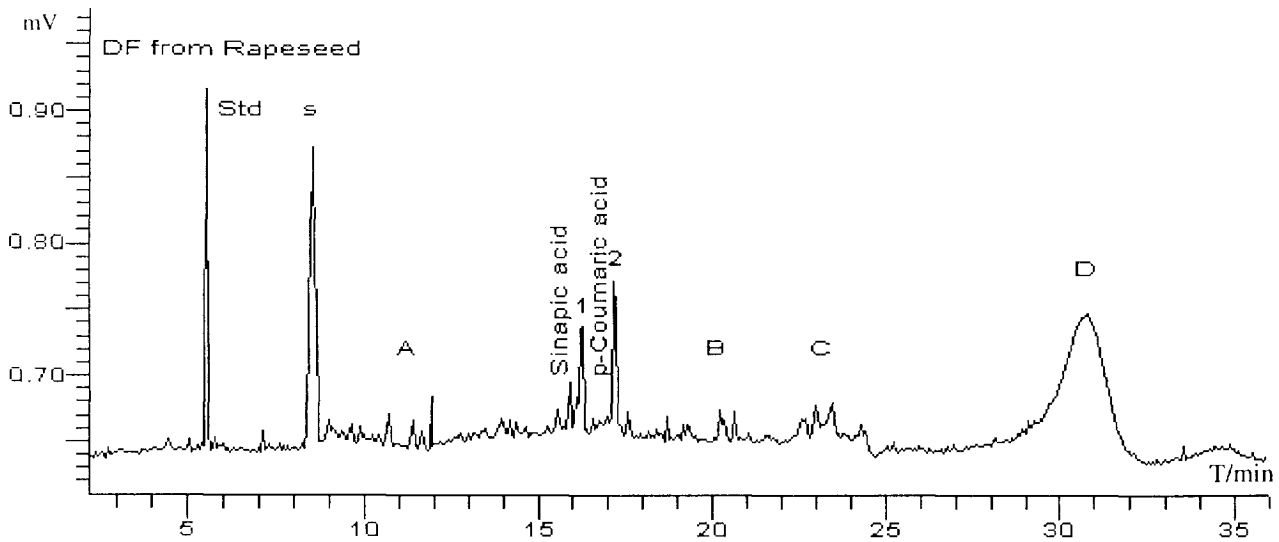

Figure 2. MECC of RS DF, SFE B analysed at condition described in Figure 1. The various peak numbers and letters are commented on in the text.

A high number of compounds were detectable in the SF extract from RS, and this was common for all the samples analysed. The identificable phenolic acids were coumaric and sinapic acid. Compounds 1 and 2 have not yet been identified, but the placement in the area of the free phenolic acids and negatively charged indole compounds indicate structures of this type. Unidentified peaks in group A is placed between the solvent front and the free phenolic acids and is possibly neutral compounds with limited interaction with the micelles. It may be polar compounds of flavonoid type, *e.g.* flavonoid glycosides, as the peaks absorb at 340 nm. Compounds in group B are expected to be

of flavonoid type as well due to absorption at 340 nm and the actual relative migration time (compare to Figure 1). Group C compounds interact strongly with the micelles and it is reasonable that the structure of these compounds could be of lignan type.

Determination of SFE extractable compounds by UV or UV-Vis detection only accounts for a minor part of the total mass as major part of the extracts consists of constituents without chromophores. TLC analyses of SFE extracts have earlier revealed high content of oil, fatty acids and dolichol-like compounds in SFE A extracts, whereas high content of phospholipids were detected in SFE B (Andersen *et al.*, 1997).

In general, the pattern described for RS DF, SFE B could be recognised in DF extracts of the other rapeseed fractions, although the relative levels vary. SFE A generally contained less of all groups of UV-Vis absorbing compounds.

CONCLUSION

SFE proved to be an effective tool in combination with UV-Vis spectroscopy and MECC for characterisation of DF associated non-carbohydrate compounds. From the gravimetric data it is seen that the total amount of DF associated compounds found by SFE vary from 7-19 % of the DF dry matter. However, there still remains some work in finding the optimal extraction time and conditions for SFE, and this also goes for the final identification of the peaks obtained by MECC. In interpretation of the results, it is important to keep in mind, that the extracted compounds are cell wall (membrane) constituents (Bjergegaard *et al.*, 1997) but they could as well be adsorbed to the DF during isolation, and this association may thus not be characteristic for the intact DF fraction in the plant. The adsorption properties of DF are on the other hand a topic of high scientific interest. DF from the various rapeseed fractions investigated differed more in amount than type of associated compounds, however, comparison to DF fractions from other plant groups (cereals, legumes) showed greater variation.

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