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Dietary fibres and associated compounds in rape seed and biorefined rape seed products compared to DF in pea*

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ABSTRACT

Dietary fibres (DF) – their chemical composition and structure, physico-chemical properties, and physiological effects – comprise and area of increasing scientific interest. Progress in this research is, however, rendered somewhat difficult by the disagreement concerning demarcation of the DF concept. In the present study, a physiological based method of analysis for determination of total DF (TDF) corresponding to the sum of insoluble DF (IDF) and soluble DF (SDF) have been used as an initial step in studies of DF from different rape seed varieties and biorefined fractions of double low rape seed compared to DF from peas.

A comprehensive characterization of DF from rape seed and peas have been performed, with determination of the neutral monosaccharide composition by different techniques as a first step. The yield of sugars, especially glucose, was found highly dependent on the procedure used for hydrolysis of the polysaccharides. The composition of the polysaccharide fractions also differed according to the type of plant material. This diversity was further emphasised by results from sequential extraction and separation of isolated DF leading to fractionation of DF into four groups: pectins, hemicelluloses, celluloses, and lignins. UV-VIS-spectroscopy of extracts from these groups provided useful information concerning the presence of material of non-carbohydrate origin. The hemicellulose fractions from peas and especially rape seed were shown to have the highest content of proteins and phenolics, possibly being present as strongly adsorbed or covalently bound components. The protein part of the DF fractions was further investigated by biochemical methods of analyses including affinity chromatography, electrophoresis as SDS IEF, and the presence of myrosinases was demonstrated in DF from rape seed. Peas also comprised DF associated proteins, although the level was considerable lower than found in rape seed DF. High performance capillary electrophoresis methods for determination of phenolics have been developed in order to provide an efficient tool for

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characterization of the phenolic DF components. Application of the above mentioned methods to studies of DF and fractions of DF have demonstrated the presence of appreciable amounts of protein and various types of low molecular weight compounds, especially phenolics, associated to DF.

KEY WORDS: dietary fibres, rape seed, pea

INTRODUCTION

Dietary fibres (Asp et al., 1988) are dominated by non starch polysaccharides (NSP) and polyphenolic lignin, but other indigestible components are found in close association with the NSP constituents, e.g. cell wall proteins and phenolic carboxylic acid derivatives, oligosaccharides of the raffinose family and tannins. In addition, resistant starch may also be included in the group of dietary fibre components (Asp et al., 1987). The proteins and the phenolic carboxylic acid derivatives are most important and are present in the largest amounts.

The development of gravimetric methods of analyses of dietary fibres (DF) had its beginning in the second half of the nineteenth century, with the introduction of the crude fibre or Weende method. This method implied extraction by dilute acid and alkali with subsequent isolation of the insoluble residue by filtration (Prosky and DeVries, 1992a, b). The residue contained only a minor and varying part of the DF fraction with the more soluble components being totally lost. This loss was also the shortcoming of the acid detergent fibre (ADF) and neutral detergent fibre (NDF) methods (Van Soest, 1963; Van Soest and Wine, 1967), however, the milder extraction conditions resulted in higher and more stable yields of the insoluble part of DF compared to the crude fibre method. The analytical approach of later developed gravimetric methods, using enzymes in combination with chemicals for extraction, comprised recovering of both insoluble (IDF) and soluble dietary fibres (SDF) (Asp et al., 1983). These methods have, on the other hand, been criticized for overestimation of the DF content by including components in the residue different from the traditional DF components, e.g. cell wall proteins, Maillard reaction products, tannins, low molecular weight (LMW) phenolic acid derivatives, saponins etc. Correction for protein and ash in DF is a common feature for many of the methods, however, the actual calculation procedure may vary, and various proteins are in fact constituents of plant cell walls and thereby of DF (Bjergegaard et al., 1997a).

The method used for TDF (IDF+SDF) in this work is the enzymatic gravimetric method, slightly modified from Asp et al. (1983). Moreover, a modification of the experimental procedure (dialysis) has been introduced in order to diminish the problems caused by ash in the SDF residues, and the DF have furthermore been investigated for compounds associated to the DF

fractions: pectins, hemicellulose, cellulose and lignin. The presence of these DF associated components has a great influence on the properties of DF, and it is therefore necessary to include these fibre associated compounds in the analysis and characterisation of DF for understanding of the properties DF have. The physico-chemical properties of DF include, among other, the ability of DF to hold water and to bind organic molecules, macro nutrients, micronutrients, xenobiotics and minerals (Bjergegaard et al., 1997a).

MATERIAL AND METHODS

Plant material

Seeds of double low winter rape and spring rape (*Brassica napus* L.) were obtained from Danish Plant breeding, St. Heddinge, Denmark cv. Ceres (CER), cv. Librador (LIB), (LIB1), cv. Bingo (BIN1), all yellow flowered, brown seeded rape seed), Maribo Frø, Holeby, Denmark (MRSW, white flowered, yellow seeded rape seed), and Trifolium Roskilde, Denmark (MRS and NRS; yellow flowered, brown seeded rape seed). Biorefining of NRS rape seed was performed by aqueous enzyme based processing resulting in: PRM (Protein rich meal), LIPRO (liproprotein) and rape seed hulls (NRH). This procedure has been described elsewhere (Olsen, 1988; Jensen et al., 1990; Bagger et al., 1996). Seeds of peas (*Pisum sativum* L.) were obtained from Danish Plant Breeding St. Heddinge, and from Pajbjergfonden, Dyngby, Odder, Denmark (cv. Bohatyr (BOH), cv. Solara (SOL), cv. Kelwo (KEL)). Grindsted Nutrio, Haderslev (Denmark) contributed with pea hulls (PHU). The samples were selected to represent rape seed, peas, as well as different fractions hereof, with appreciable variations in DF levels.

Chemicals and enzymes

All chemicals were of analytical-reagent grade. Water was double deionized. The enzymes used comprised a heat resistant α-amylase (Termamyl, 120 L; Novo Nordisk A/S, Copenhagen, Denmark), pepsin (2000 FIP-U/g, Cat. no. 7190, Merck, Darmstadt, Germany), and pancreatin (4 x U.S.P., Cat. no P-1750; Sigma, Montana, USA).

Analysis of dietary fibres

Development of an AOAC method (Association of Official Analytical Chemists) for determination of total dietary fibres (Prosky et al., 1984, 1985) has

been based primarily on the experience of three scientific groups (Schweizer and Würsch, 1979; Furda, 1981; Asp et al., 1983). In these methods, TDF were further divided into IDF and SDF, and in 1988, the AOAC method for determination of TDF was suggested correspondingly extended (Prosky et al., 1988). The principles of analysis comprise in all methods enzymatic treatments of the sample followed by filtration or centrifugation to obtain the insoluble residue. The soluble residue is then isolated (filtration/centrifugation) from the filtrate or supernatant by precipitation with ethanol. A preliminary chemical extraction of fat from the sample is common in all methods, whenever the fat content exceeds 5-10%. This extraction can now be performed as a fast and simple SFE procedure (Buskov et al., 1997).

Conspicuous is the total time of incubation with enzymes, which has been reduced from 38 h to 1.25 h (Table 1). High efficiency of the starch and protein digestion with short incubation times was verified by Asp et al. (1983), using Termamyl, pepsin and pancreatin and a total incubation time of 2.25 h. Termamyl, a heat-stable α-amylase free of any contaminating β-glycosidase activities, is working in the starch gelatinization step, avoiding retrogradation of solubilized starch when cooling the samples (Theander, 1983). Resistant starch (RS) is not degraded by Termamyl, and will contribute to the DF value (Asp, 1990).

The enzymatic gravimetric analysis used followed the principles described by Asp et al. (1983). The present study comprised two methods where the analytical procedure in method II comprised some extra modifications. All samples were run in duplicate, and a blank (no sample, only reagents) were included for every new series of analyses.

TABLE 1

Overview of selected steps in enzymatic gravimetric methods used for determination of DF (TF, IDF, SDF). The parenthesis in the incubation media column state whether HCl or NaOH have been used for regulation of pH

Method	Enzymes	Incubation			
		media	pH	time min	temperature °C
Asp et al. (1983)	Termamyl	0.1 M phosphate	6.0	15	100
	Pepsin	+ water (HCl)	1.5	60	40
	Pancreatin	+ water (NaOH)	6.8	60	40
Prosky et al. (1988)	Termamyl	0.08 M phosphate	6.0	15	100
	B. subtilis protease	0.08 M phosphate + NaOH	7.5	30	60
	Amyloglucosidase	+ 0.33 M HCl	4.5	30	60

Analysis of DF; Method I

Seeds were milled in a laboratory mill, whereas PRM and PHU were milled at the place of production. Moisture content was determined by drying to constant weight (3 h at 105°C).

Fat extracted, milled seeds (0.5 g) were weighed into 100 ml Erlenmeyer flasks with magnet and thoroughly suspended in sodium phosphate buffer (25 ml, 0.1 M, pH 6.0). Termamyl (100 μl) was added and the covered flasks were placed in a boiling water bath for 30 min. The samples were mixed manually at 5 min intervals. HCl (20 ml, 0.2 M) was added after cooling to room temperature, and pH was adjusted to 1.5 with HCl (1.0 M) or NaOH (1.0 M). Pepsin (100 mg) was added and the samples were incubated in water bath with shaker (60 min; 40°C). Magnetic stirring of the samples was performed after 30 min. NaOH (10 ml, 0.55 M) and sodium phosphate (10 ml, 0.2M, pH 6.8) were added and pH was adjusted to 6.8 with 1.0 M HCl or 1.0 M NaOH. Pancreatin (100 mg) was added and the samples were incubated in water bath with shaker (60 min; 40°C). Magnetic stirring of the samples was performed after 30 min. CH_3COOH (5 ml, 0.94 M) was added and pH was adjusted to 4.5 ± 0.1 with 1.0 M HCl.

IDF was isolated by filtration on a filter crucible (porosity 40-100 μm) with celite (0.5g), and the residue (IDF) was washed with 2x10 ml water. Filtrates and water washing were saved for determination of SDF. The IDF residues were further washed with 2x10 ml 96% ethanol and 2x10 ml acetone, and the residues were dried and weighed.

SDF was precipitated from the filtrate+water washing with ethanol (4 volumes of 96% ethanol, 60°C, 60 min) before the precipitate was isolated by filtration (wash with 2x10 ml 80% ethanol; 2x10 ml 96% ethanol; 2x10 ml acetone) or centrifugation, dried and weighed.

Analysis of DF; Method II

The analytical procedure was identical to that of method I to the point where filtration start. The changes in the filtration procedure include omission of celite from the crucibles used for filtration of IDF. With respect to SDF, the precipitation step was replaced by overnight dialysis (cut-off-value for dialysis bags: 12000-14000 g/mol) of the combined filtrate, followed by freeze drying. N and ash analyses were performed on the weighed residues. N and ash analyses on blank samples within the different methods followed the principles for the actual method described above.

Analyses of N and ash

The content of N was determined according to the Dumas principle, with N-oxides, produced by combustion at high temperature (about 1000°C) in an oxygen atmosphere, being reduced to N_2 by the passage of a copper-containing column. N_2 was detected in a thermal conductivity cell and the factor 6.25 was used for calculation of corresponding protein content. The apparatus used was a Carlo Erba NA 1500 Automatic Nitrogen Analyzer. Ash was determined according to the standard method (AOAC, 1965).

Fractionation of dietary fibres

The fractionation scheme used in the present study divided the isolated DF into four fractions named pectic material (PEC), hemicelluloses (HEM), cellulose (CEL), and lignins (LIG). The fraction names refer to the actual extraction conditions.

(A) PEC: Extraction of pectic polysaccharides may be performed in several ways including use of hot water, cold water and hot or cold aqueous solutions of different chelating agents (Selvendran et al., 1985; Fry, 1988).

PEC was obtained as the supernatant after extraction of IDF or SDF (1 g) with water (25 ml) for 2 h at 70°C in a shaking waterbath. The sediment remaining after cooling and centrifugation (34000 x g; 20 min) was freeze dried, weighed, and used in (B). A fraction of supernatant (5 ml) was kept at −20°C for further analyses. The rest (known volume) was dialysed against water overnight (5°C) and another 5 ml was saved (−20°C). The remaining dialysed supernatant (known volume) was freeze dried, weighed, and saved in desiccator.

(B) HEM: Hemicelluloses comprise polysaccharides obtained by selective extraction with aqueous alkali after the water soluble pectic substances have been removed (Dreher, 1987). The extraction can be performed with a range of alkali strengths, and hemicellulose A, B, and C are examples of different subfractions of hemicelluloses. Hemicellulose A appears as a precipitate, when neutralizing or slightly acidifying the base extract (weak alkali) with acetic acid, and hemicellulose B is obtained when adding ethanol to 70-80% in the pH adjusted extract. Hemicellulose C is extracted by use of strong alkali (Dreher, 1987; Brett and Waldron, 1990; Southgate, 1991).

HEM was obtained as the supernatant after extraction of the sediment from (A) with 2.0 M NaOH (25 ml) under N_2 for 2 h in a boiling waterbath with occasional shaking. The sediment remaining after cooling and centrifugation (34000 x g; 20 min) was freeze dried, weighed, and used in (C). The supernatant was made weakly acidic (3-4 ml acetic acid), and 5 ml of the pH adjusted supernatant was kept at −20°C for further analyses. The supernatant (known

volume), was dialysed overnight (5°C). Sedimented material was collected by centrifugation (3000 x g; 3 min), freeze dried, weighed, and kept in desiccator. The dialysed supernatant (5 ml) was kept at −20°C for further analyses. The remaining dialysed supernatant (known volume) was freeze dried, weighed and kept in desiccator.

(C) CEL was obtained as the supernatant after dissolving the sediment from (B) in 12.0 M H_2SO_4 (1 ml, 35°C) under occasional mixing. After 1 h, the mixture was diluted with 11 ml H_2O to give a 1.0 M H_2SO_4 solution, and continued extraction and hydrolysis were performed in a boiling waterbath under reflux for 18 h. The sediment remaining after cooling and centrifugation (table centrifuge; 3000 x g; 3 min) was freeze dried, weighed, and kept in desiccator (D). The supernatant (known volume) was neutralized with saturated $Ba(OH)_2$ and centrifuged to remove $BaSO_4$. Neutralized supernatant (5 ml) was kept at −20°C for further analyses, and the rest (known volume) was freeze dried, weighed, and kept in desiccator.

(D) LIG was obtained as the residue after acid extraction as described under (C). LIG is identical to Klason lignin.

The dialysis described was performed by use of dialysis bags with a cut-off-value ranging from 12000-14000 g/mol. The colour of the different supernatants was noted prior to freeze drying.

Thymol-sulphuric acid analysis of fractionated material

The content of total carbohydrates (expressed as glucose) was determined for the PEC and HEM supernatants after dialysis and for the neutralized non-dialysed CEL supernatant by the thymol-sulphuric acid method.

For samples of isolated DF 2-3 mg (known weight) were mixed with H_2SO_4 (1 ml; 77%), and allowed to stand for 10 min with occasional mixing. Water (4 ml) was then added carefully. After 5 min, 100 µl of this solution was transferred to acid washed test tubes (H_2SO_4) together with 400 µl water, 50 µl 1% thymol solution (ethanol basis), and 3500 µl 77% H_2SO_4. In the reference sample, 100 µl probe was replaced by 100 µl water. A standard curve was prepared from a glucose stock solution (0.1 mg glucose/ml). The mixtures were placed covered in a boiling waterbath for 35 min, with mixing after 10 and 20 min. Samples were cooled for 10 min. The absorbance (undiluted samples) was measured in quartz cuvettes at 505 nm.

Hydrolysis of polysaccharides

Hydrolysis of IDF and SDF was performed according to three different methods.

Samples for GLC were hydrolysed with TFA, according to the principles of Albersheim et al. (1967). TFA (0.2 ml; 2.0M) was added to 10 mg of isolated DF together with 1 mg myoinositol, serving as internal standard. Hydrolysis was performed in a sealed tube at 121°C for 1 h. TFA was removed from the hydrolysate by evaporation to dryness.

GLC analysis was performed as described elsewhere (Bjergegaard et al., 1995, 1997b).

Samples for HPLC (70 mg) were boiled under reflux in 1.0 M H_2SO_4 (5 ml) for 16 h. After cooling, the hydrolysate was centrifuged for 3 min (3000 g) and the supernatant collected. Washing of the sediment with water (1 ml) was performed 3 times, with recentrifugation and collection of supernatant after each washing step. The mixed hydrolysate was neutralised with a saturated $Ba(OH)_2$ solution and centrifuged to removed precipitated $BaSO_4$. The final volume of the neutralised supernatant was adjusted to 40 ml and 10 ml hereof was evaporated to dryness prior to further purification.

The hydrolysates were purified on reversed phase C-18 HPLC material (Bondapak C-18; 75μM, Waters Associates) packed as 100 μl of a material: methanol suspension (1:1) into glass wool containing pipette tips. The methanol was washed out gradually, increasing the proportion of water in the water-methanol solutions used for washing. About 200 μl sample (redissolved in 1 ml water) was transferred to the column, and the effluent was discarded to avoid dilution. Sample (100 μl) was loaded, and the effluent collected into HPLC vials.

Samples for HPCE were hydrolysed according to the method of Englyst and Cummings (1988). H_2SO_4 (1 ml; 12.0M) was added to 100 mg of isolated DF, mixed, and left for 1 h at 35°C in a shaking waterbath. In addition, manual shaking was performed after 30 min. Water (11 ml) was added, and the sample was boiled under reflux for 2 h with occasional mixing. The resulting hydrolysate was treated as described for the HPLC samples (*vide infra*). The neutralized supernatant was made up to a known volume (about 60 ml), and 15 ml hereof was evaporated to dryness.

Purification prior to HPCE analysis was performed according to the principles of Bjerg et al. (1984). A strongly acidic cation exchanger (Dowex 50w x 8; 200-400 mesh (H^+)) was connected to a strongly basic anion exchanger (Dowex 1 x 8; 200-400 mesh (OH^-)). Ion exchange material:water suspension (500 μl; 1:1) were packed into tubes (1 ml) with outlet discs of silica material, and placed in a vacuum manifold (Supelco Inc., Pennsylvania, USA). The columns were washed with minimum 10 ml water. Samples (500 μl of sample redissolved in 1 ml water) were transferred to the upper column (cation exchanger) and allowed to pass into the material of the connected columns. Elution was performed with 15 ml water. The effluent was evaporated to dryness in a rotation evaporator, redissolved in 500 μl or 1000 μl water and used for HPCE analysis.

HPLC and HPCE analyses were performed as described elsewhere (Frias et al., 1996; Bjergegaard et al., 1997b).

Electrophoresis

SDS-PAGE and IEF were performed as described in Bjergegaard et al. (1997b).

RESULTS AND DISCUSSION

Corrections for protein and ash

Filtration did not constitute any problem in method I, whereas the omission of celite in method II prolonged the time for filtration of IDF. All samples were, however, possible to filtrate within 2-3 h. The level of IDF and SDF (uncorrected values), obtained by method I and II, respectively, are shown in Table 2 for some of the samples.

TABLE 2

Uncorrected level of IDF and SDF in selected pea and rape seed samples given as mean values (double determination). The figures in parenthesis are relative standard deviations, %

Sample	IDF		SDF	
	Method I	Method II % of DM	Method I	Method II % of DM
BOII	17.5 (0.6)	15.7 (10.6)	9.4 (5.7)	25.4 (0.1)
PHU	84.5 (1.1)	83.9 (1.7)	15.2 (9.4)	27.7 (3.4)
CER	30.2 (9.6)	36.0 (1.7)	9.4 (1.2)	14.8 (7.4)
MRSW	23.5 (7.6)	36.7 (6.3)	10.6 (23.0)	17.7 (12.8)
PRM	25.6 (0.3)	30.4 (0.8)	9.5 (4.2)	21.4 (3.9)
LIPRO	22.9 (2.1)	23.8 (3.2)	7.3 (2.8)	–

Method II gave considerably higher uncorrected SDF levels, compared to the results obtained using the method I including traditional filtration and precipitation steps. For IDF, slightly higher values were seen only for the rape seed samples.

The calculated contents of protein and ash in the residues are shown in Table 3. The protein level in SDF was found to be considerably higher with method II, compared to method I, whereas the protein level in IDF was comparable in size for the two methods. Ash was generally not detected in IDF. This was also true for SDF determined by method II, whereas ash constituted up to 50% of the

TABLE 3

The content of protein (P) and ash (A) in the residues from a double determination (1 and 2), %

Sample		Method I				Method II			
		1		2		1		2	
		P	A	P	A	P	A	P	A
BOH	IDF	18.8	0.0	18.8	0.0	10.3	0.0	13.7	0.0
PHU	IDF	2.0	0.0	1.9	0.0	1.5	0.0	1.5	0.0
CER	IDF	18.9	0.0	17.2	3.4	16.4	0.0	14.5	0.0
MRSW	IDF	22.7	0.0	24.6	0.0	17.6	0.0	19.3	0.0
PRM	IDF	50.0	0.0	50.8	0.0	48.2	0.0	51.6	0.0
LIPRO	IDF	–	–	–	–	17.0	1.2	19.3	0.0
BOH	SDF	17.7	26.6	19.0	21.4	30.8	0.0	31.8	0.0
PHU	SDF	8.9	30.9	9.9	22.9	20.9	0.0	25.2	0.0
CER	SDF	11.5	47.8	11.7	47.2	36.4	0.0	39.7	0.0
MRSW	SDF	14.4	32.9	10.8	49.8	36.7	0.0	39.3	0.0
PRM	SDF	11.7	47.1	12.5	43.6	46.7	0.0	50.0	0.0
LIPRO	SDF	–	–	–	–	39.7	0.0	42.3	0.0

residues, when method I was used, and considerable variation was seen for these ash content determinations.

The level of DF obtained after correction for ash and protein in the residues is shown in Table 4. RSD values for SDF determination by method II were found to be high owing to the high correction values for proteins, whereas for IDF, the RSD values ranged from 0.1 to 16.1%. The level of IDF and SDF for rape seed

TABLE 4

Corrected levels of IDF and SDF in selected pea and rape seed samples given as mean values (double determination). The figures in parenthesis are relative standard deviations, %

Sample		Method	
		I	II
			% of DM
BOH	IDF	13.8 (1.3)	12.4 (16.4)
PHU	IDF	81.6 (1.4)	79.9 (0.6)
CER	IDF	22.4 (8.8)	29.1 (2.0)
MRSW	IDF	18.7 (8.5)	28.2 (5.3)
PRM	IDF	12.3 (0.2)	13.8 (1.0)
BOH	SDF	3.6 (1.1)	8.2 (7.0)
PHU	SDF	7.9 (2.0)	9.8 (13.6)
CER	SDF	2.7 (0.7)	2.8 (32.0)
MRSW	SDF	3.4 (3.5)	3.4 (31.4)
PRM	SDF	2.6 (0.1)	3.0 (18.1)

samples varied, dependent on the method used for calculation/determination. The level of IDF in the pea samples varied only little within double determinations and was also found relatively constant between the different methods.

The enzymatic gravimetric methods for determination of DF have evolved to their present state due to a comprehensive work within the last 10-15 years. The physiological approach of the methods has often been criticized for leading to a residue, which exact composition in outline is unknown to the analyst, and which contains different components in addition to the traditional DF constituents (NSP and lignins) (Bjergegaard et al., 1997a). This is, however, a question of definition, and should not be blamed the methods. Anyhow, a clarification regarding the correction for protein is needed within the nearest future. Concerning the ash correction, being complicated by ash bound water, the newest modification to the AOAC procedure by Lee et al. (1992) seems to have reduced the problem by decreasing co-precipitation of buffer-salts. However, it is a question whether this modification is desirable from a physiological point of view. Rather, new methods for recovering of SDF, without contemporary recovering of ash, should be developed, and in this connection attention should be given to possible variation in the SDF level obtained according to different analytical principles. An evaluation of the type of polymers recovered as SDF by dialysis but excluded by the precipitation procedure should thus be performed as a first step in order to decide a possible inclusion of these polymers in the SDF fraction. Whether SDF then have to be determined directly or by difference calculation should depend on the precision of the actual method.

The procedure used for correction of results has been somewhat overlooked in the enzymatic gravimetric methods. In the present study, it has been shown, that the way of calculating the ash and protein content from the same set of data affected the precision of the methods, although the mean values remained within the same range. From an analytical point of view, correction for ash and protein in a residue should not be performed on the basis of results from analyses of another residue, as is the common procedure at present.

Hydrolysis of polysaccharides

Results obtained by analyses for individual sugars reflect especially the methods of polysaccharide hydrolysis. The procedures used for hydrolysis prior to GLC give an underestimation of glucose from polysaccharides as cellulose which only is slightly soluble in the solvent system used for hydrolysis. The techniques described by Englyst and Cummings (1988) which were used prior to HPCE give higher results for glucose from such slightly soluble β-glucans, but this system creates problems concerning the stability of other carbohydrates.

TABLE 5

Relative proportion (%) of monosaccharides in DF isolated from rape seed and peas. Determination of individual monosaccharides was performed by GLC (double determination, mean values given). Figures in parenthesis are standard deviations, %

Sample		Fucose	Mannose	Galactose	Arabinose	Xylose	Glucose
		relative proportion, %					
SOL	IDF	1.2 (0.11)	1.2 (0.60)	6.6 (0.06)	25.9 (2.13)	10.9 (1.55)	54.1 (4.45)
	SDF	–	–	–	–	–	–
KEL	IDF	1.5 (0.11)	0.8 (1.06)	10.7 (0.12)	35.0 (0.02)	14.8 (1.53)	37.3 (0.50)
	SDF	2.5 (0.02)	2.4 (0.11)	41.2 (0.15)	5.4 (0.30)	1.5 (0.19)	47.0 (0.26)
BOH	IDF	0.9 (0.03)	0.5 (0.01)	7.9 (0.24)	19.9 (0.12)	11.4 (1.09)	59.5 (1.47)
	SDF	1.6 (0.16)	1.5 (0.02)	18.9 (0.70)	3.2 (0.13)	0.7 (0.08)	74.2 (0.32)
BOH1	IDF	1.0 (0.02)	0.3 (0.43)	12.7 (0.59)	28.5 (0.09)	11.1 (0.66)	46.4 (3.23)
	SDF	1.7 (0.10)	1.4 (0.02)	16.8 (0.45)	3.6 (0.08)	0.6 (0.02)	75.8 (0.44)
BOH2	IDF	1.1 (0.16)	5.2 (0.03)	9.5 (1.12)	29.3 (1.20)	11.0 (2.06)	43.9 (0.07)
	SDF	1.5 (0.04)	1.4 (0.03)	17.4 (0.08)	4.5 (0.04)	0.7 (0.02)	74.4 (0.14)
PHU	IDF	2.1 (0.05)	trace	7.1 (1.10)	17.4 (4.20)	60.5 (3.06)	12.9 (0.00)
	SDF	5.5 (0.05)	3.9 (0.15)	5.5 (0.70)	50.9 (0.81)	20.9 (0.02)	13.2 (0.01)
MRS	IDF	3.3 (0.04)	2.4 (3.38)	25.1 (1.08)	33.7 (1.25)	15.7 (0.48)	19.8 (0.52)
	SDF	3.5 (0.25)	5.7 (2.17)	22.9 (0.79)	55.6 (1.42)	6.3 (0.01)	6.0 (0.20)
NRS	IDF	3.5 (0.06)	2.2 (0.14)	22.4 (1.02)	39.6 (1.83)	18.3 (0.88)	14.1 (0.01)
	SDF	6.5 (0.48)	8.8 (3.00)	26.0 (0.40)	40.5 (1.63)	8.3 (1.82)	10.0 (0.53)
PRM	IDF	4.1 (0.17)	12.6 (2.45)	31.5 (0.89)	17.4 (0.78)	13.2 (0.54)	21.1 (0.07)
	SDF	6.2 (0.07)	11.6 (0.59)	38.4 (0.51)	22.3 (0.03)	13.3 (0.04)	8.2 (0.02)
NRH	IDF	3.3. (0.83)	2.7 (0.09)	33.3 (6.18)	39.4 (3.70)	10.7 (0.56)	10.7 (1.00)
	SDF	7.5 (0.23)	5.0 (0.28)	27.7 (0.73)	37.4 (0.39)	12.4 (0.55)	10.0 (0.16)

Table 5 shows results from GLC determination of individual monosaccharides in hydrolysates from IDF and SDF.

The GLC analysis (Table 5) demonstrated a low content of fucose and mannose in pea DF, the level being highest for PHU SDF (9.4%). The level of glucose found by GLC was low, especially for IDF, compared to the data obtained by HPLC, and consequently, the apparent proportion of the other monosaccharides increased. Apart from small deviations, the overall trend for IDF was, however, on the whole similar in GLC and HPLC. For SDF, arabinose was demonstrated in the intact seed samples (3.2-5.4%). Otherwise, the GLC data corresponded well to the HPLC results, except for PHU, which appeared shown to have a completely different and more varied composition when analyzed by GLC.

The monomeric composition of rape seed DF was shown to be different from that of pea DF (Table 5). In rape seed, fucose and mannose were present in higher levels, whereas the glucose content in general was a factor 2-7 lower, the reduction being greatest in the SDF fraction. The relative proportion of glucose in DF from rape seed and pea hulls was, however, very similar. The quantitatively dominating sugars in rape seed DF were galactose and arabinose, the sum constituting 49-73% and 61-79% of IDF and SDF, respectively. Generally, the level of arabinose was highest, apart from in PRM, being dominated by galactose.

Fractionation of dietary fibres

Studies of the plant cell wall matrix polysaccharides comprise certain difficulties as emphasized by Brett and Waldron (1990). The general experimental approach include purification of the plant cell wall followed by sequential extraction (fractionation) and physical/chemical studies of the extracted material. The two main problems in connection with fractionation are 1. that a certain number of bonds must be broken in order to extract components from the cell wall, leading to incorrect conclusions about the chemistry of DF polysaccharides and 2. that the extraction of components may result in only partial recovery of a particular type of polysaccharide, and hereby lead to some discrepancy between the classification of polysaccharides, based on solubility properties and chemical composition, respectively.

Evaluation of the data obtained from fractionation of IDF and SDF from rape seed and peas, respectively, showed a varying and very complex picture for the relationship of PEC, HEM, CEL, and LIG, depending not only on the DF source but also on the actual calculation mode (Table 6).

The difference values obtained as Dif. (Table 6) are figures where the weight of a fraction is based on disappeared material. Although the preparation of plant material prior to fractionation has comprised an isolation of IDF and SDF, the Dif. values may cover up compounds not generally included in the DF fraction. An example is the occurrence of co-precipitated buffer salts in SDF, and this illustrates very well the problem with the enzymatic gravimetric method when used without dialysis (vide supra). Comparison of the weight of fractions obtained as Dif. and Abs. (Table 6) shows that Dif. values were a factor 3.1-9.5 (5.2 in average) higher for non-dialysed SDF, whereas the corresponding factor for SDF dialysed prior to the fractionation was 1.5-2.2 (1.9 in average). This emphasize, that a part of "pectic material" extracted from SDF by water actually consist of ash.

TABLE 6

Proportion between pectic material, hemicelluloses, cellulose, and lignins in DF isolated from rape seed and peas. Dif., Abs. and Thy values are obtained by the procedure described in the text. SDF from MRS, NRS, PRM and NRH were dialysed prior to fractionation, %

		Pectic material			Hemicelluloses			Cellulose			Lignins		
		Dif	Abs	Thy	Dif	Abs	Thy	Dif	Abs	Thy	Dif	Abs	Thy
		% of DF											
MRS	IDF	27.3	8.5	2.9	33.3	36.3	41.8	32.6	39.5	41.0	6.7	15.6	14.3
	SDF	35.6	27.5	70.0	19.9	15.0	0.5	33.3	40.8	3.3	11.1	16.8	26.1
MRSW	IDF	17.6	1.8	2.2	27.8	29.1	43.8	35.5	49.5	16.5	19.0	19.6	37.6
	SDF	72.9	51.5	89.8	9.5	25.0	6.4	17.7	23.3	3.8	0.0	0.0	0.0
NRS	IDF	20.7	6.4	5.3	32.3	32.5	38.5	35.7	42.0	30.2	11.3	19.0	26.1
	SDF	47.4	48.4	97.6	11.2	19.5	0.0	40.6	30.4	0.0	0.9	1.6	2.3
PRM	IDF	18.2	6.2	4.3	56.0	64.2	18.6	17.7	11.9	56.5	8.1	17.7	20.7
	SDF	43.2	40.4	76.6	7.6	8.8	0.0	33.5	28.4	0.0	15.7	22.4	23.5
NRH	IDF	5.7	2.4	3.6	40.0	35.0	15.5	35.9	37.2	55.4	18.5	25.5	25.5
	SDF	40.6	31.2	76.4	5.6	16.2	1.7	44.9	36.3	0.2	8.9	16.4	21.5
LIB	IDF	30.5	2.8	2.2	27.5	29.9	34.8	24.4	42.5	27.2	17.6	24.8	35.8
	SDF	68.7	42.6	90.9	11.8	24.5	7.4	19.5	332.8	1.7	0.0	0.0	0.0
LIB1	IDF	17.4	2.3	2.0	26.6	28.8	41.3	37.4	46.2	19.2	18.6	22.8	37.5
	SDF	76.9	47.5	86.1	9.5	25.0	10.8	13.6	27.5	3.0	0.0	0.0	0.0
BIN1	IDF	14.7	1.9	1.9	25.3	28.3	31.8	35.0	41.0	24.2	25.0	28.8	42.1
	SDF	75.1	57.9	91.8	10.2	22.9	7.6	14.7	19.2	0.6	0.0	0.0	0.0
SOL	IDF	13.3	11.5	12.0	43.9	33.3	46.6	41.4	52.7	38.0	1.4	2.5	3.4
	SDF	91.2	84.9	99.7	3.2	14.2	0.0	5.7	0.5	0.0	0.1	0.4	0.3
KEL	IDF	20.0	3.5	3.1	22.2	25.1	40.3	45.6	52.1	36.9	12.2	19.3	19.7
	SDF	91.4	61.8	92.6	5.1	37.6	3.7	3.5	0.6	3.7	0.0	0.0	0.0
BOH	IDF	28.1	6.1	3.5	34.6	56.7	43.5	33.9	29.0	48.0	3.4	8.1	5.0
	SDF	95.0	65.0	67.5	2.4	24.5	15.1	1.3	1.6	9.6	1.3	8.6	7.9
BOH1	IDF	18.5	4.4	4.3	32.1	29.6	42.5	33.6	40.6	26.2	15.8	25.4	27.1
	SDF	92.3	64.2	89.8	5.3	34.8	6.1	2.4	1.0	4.1	0.0	0.0	0.0
BOH2	IDF	5.4	3.8	4.1	45.5	30.5	44.3	30.3	34.3	19.2	18.8	31.3	32.3
	SDF	92.6	50.5	92.3	3.9	28.9	5.1	3.4	20.7	2.6	0.0	0.0	0.0
PIIU	IDF	7.8	1.7	3.3	10.6	11.4	11.5	52.8	56.7	59.1	28.8	30.2	26.1
	SDF	86.0	77.3	84.9	7.9	14.7	7.1	3.7	1.0	2.4	2.5	7.0	5.6

Dif. = The weight difference between starting material and dried sediment after corresponding extraction. Abs. = The weight of the freeze dried supernatants. Thy. = results from Thymol-H_2SO_4 analysis.

Non-carbohydrate content in fractionated DF

The absorption at 280 nm can in part for dialysed samples be considered as a relative measure for protein (Table 7), whereas low molecular weight compounds are removed by dialysis. As seen, the values are highest for the non-dialysed PEC fraction in SDF and for the non-dialysed HEM fraction in IDF (Tables 7 and 8). This trend was common for rape seed and peas, however, the 280 nm absorption for rape seed IDF was in average a factor 5 higher than for pea IDF. A considerably higher absorption in IDF compared to SDF was also revealed from the sum of absorptions. For peas, this difference constituted a factor 2-5, whereas in rape seed, the corresponding factor was 4-15.

The absorption level within the various fractions in the different pea samples generally showed limited variation, with the exception of PHU IDF, apparently having a very high protein content in the CEL fraction. The presentation of data

TABLE 7

Content of protein in pectic material, hemicelluloses, and cellulose (DM basis) from pea IDF and SDF. The maximum value within the individual DF sources are shown in italics bold. The figures in percentage express the amount of UV absorbent material retained after dialysis of the supernatants. b.d. = before dialysis; a.d. = after dialysis; n.d. = non-dialysed. Missing observations are marked by "–". Data are relative values, denoted mmol based on use of $\varepsilon = 1500\ M^{-1}$ for protein in $E = \varepsilon \cdot c \cdot l$

Sample		Protein content, mmol/g isolated DF							
		Pectic material			Hemicelluloses			Cellulose	SUM
		b.d.[1]	a.d.[2]	%	b.d.[1]	a.d.	%	n.d.[2]	
SOL	IDF	0.14	0.05	35.0	***0.52***	0.08	16.3	0.09	0.74
	SDF	***0.33***	0.21	63.9	0.04	0.01	11.6	0.01	0.38
KEL	IDF	0.12	0.03	25.2	***0.51***	0.07	14.3	0.14	0.77
	SDF	***0.34***	0.13	38.2	0.02	0.01	31.8	0.00	0.36
BOH	IDF	–	0.02	–	***0.38***	0.05	14.1	0.18	0.59*
	SDF	–	***0.09***	–	0.02	0.00	16.7	0.00	0.11*
BOH1	IDF	0.11	0.03	25.7	***0.46***	0.08	17.2	0.08	0.65
	SDF	***0.29***	0.11	37.5	0.02	0.01	31.8	0.01	0.32
BOH2	IDF	0.10	0.02	20.2	***0.53***	0.10	19.5	0.08	0.71
	SDF	***0.30***	0.11	37.5	0.03	0.01	18.2	0.00	0.33
PHU	IDF	–	0.01	–	0.33	0.05	15.0	***0.41***	0.76*
	SDF	–	***0.13***	–	0.05	0.01	12.5	0.01	0.18*

[1] values used for calculation of the sum

[2] values used for calculation of the sum if missing observations before dialysis

* sum calculated partly on the basis of values from 2

TABLE 8

Content of protein in pectic material, hemicelluloses, and cellulose (DM basis) from rape seed IDF and SDF. Comments and footnotes as in Table 7

Sample		Protein content, mmol/g isolated DF							
		Pectic material			Hemicelluloses			Cellulose	SUM
		b.d.[1]	a.d.[2]	%	b.d.[1]	a.d.	%	n.d.[1]	
MRS	IDF	0.20	0.04	21.8	*1.08*	0.20	18.0	0.05	1.34
	SDF	*0.24*	0.15	63.6	0.03	0.04	–	0.03	0.30
MRSW	IDF	0.09	0.02	24.9	*2.94*	0.46	15.7	0.04	3.07
	SDF	*0.18*	0.13	72.8	0.08	0.01	14.7	0.01	0.28
NRS	IDF	0.20	0.05	25.2	*2.52*	0.59	26.2	0.07	2.51
	SDF	*0.40*	0.30	75.8	0.02	0.07	29.7	0.02	0.44
PRM	IDF	0.20	0.07	37.6	*1.48*	0.25	16.6	0.03	1.71
	SDF	*0.33*	0.29	88.5	0.06	0.01	9.8	0.02	0.40
NRH	IDF	0.09	0.03	33.8	*4.82*	1.39	28.7	0.07	4.97
	SDF	*0.21*	0.12	57.5	0.10	0.02	14.7	0.02	0.33
LIB	IDF	0.15	0.03	22.0	*2.06*	0.46	22.1	0.05	2.26
	SDF	*0.19*	0.13	70.4	0.10	0.02	15.9	0.01	0.30
LIB1	IDF	0.13	0.03	26.4	*2.40*	0.59	24.5	0.05	2.58
	SDF	*0.30*	0.22	74.3	0.14	0.01	7.9	0.01	0.45
BIN1	IDF	0.11	0.04	32.2	*2.69*	0.59	21.9	0.05	2.85
	SDF	*0.30*	0.25	82.5	0.10	0.01	13.3	0.01	0.41

as mmol/g isolated DF do, however, not take into account, that the actual amount of CEL in IDF from PHU was about two times higher compared to the other pea samples, making the difference per g/cellulose less marked. This has to be taken into consideration in evaluation of the data.

Differences in the absolute amount of material in the various rape seed fractions (Table 8) did, however, only explain a minor part of the variations obtained in absorption here. The HEM fraction of NRH IDF differed in having an extremely high absorption at 280 nm, and this indication of a high protein content in the HEM fraction was confirmed by data from isoelectric focusing of the dialysed freeze dried material.

The absorption retained after dialysis was calculated for PEC and HEM supernatants, respectively, and gives an idea of 1. the type (size) of UV absorbent material and 2. its association to the other DF components in the fractions. About 75% of the UV absorbent compounds disappeared from the PEC supernatant of IDF after dialysis, indicating, that the main part of this material was LMW compounds present in free solution or loosely adsorbed to com-

ponents in this fraction. Note that part of these compounds not necessarily have been associated to pectic material originally, but as well could have been extracted from other DF components to the PEC supernatant by the 2 h water treatment. In rape seed SDF, the main part of the UV absorbent material was retained (60-90%), whereas in pea, the corresponding figures varied from 40-60%. This difference are not alone brought about by dialysis of rape seed SDF prior to fractionation, as also non-dialysed rape seed samples showed a higher retainment percent.

The UV absorbent material in the HEM supernatants must originally have been present as strongly adsorbed or covalently bound components, as more readily extractable material are expected to occur in the PEC fraction as described above. 20% or less of the UV absorbent material was retained in the dialysed HEM supernatant of IDF as well as SDF, and this indicates, that the dominating association type for these compounds was alkali-labile, e.g. ester bonds. Actually, it is not possible to decide the origin of this ester bound material, which could be from the LIG or CEL fraction as well as from the HEM fraction. The possible presence of compounds of non-protein origin with absorption at 280 nm emphasize, that the calculated protein content is an arbitrary figure. So, the information from UV-VIS-spectra should preferably be used as a first hint of the occurrence of compounds of non-carbohydrate origin in the DF fraction. This has then to be followed up by further investigations of the compounds present. The occurrence of ester bound benzoic acid derivatives has been demonstrated in IDF from rape seed by use of HPCE (Bjergegaard et al., 1997b). Ether bound or carbon-carbon bound substituents will not be released by the alkali treatment.

The absorption at 320 nm (Tables 9 and 10) followed in outline the same pattern as described above for 280 nm, however, the difference in the total sum of absorption between IDF and SDF was even more distinct (5-12 and 8-30 times higher for IDF in peas and rape seed, respectively).

Comparison of the sum of absorption at 320 nm of PHU and NRH IDF showed a 12 times higher absorption for rape seed hulls, whereas the difference for the whole seeds was less marked (4 times higher for rape seed). The very high absorption in the HEM fraction of NRH IDF may be partly mediated by the dark colour of the supernatant, probably caused by an oxidation of phenolic material associated to the hemicellulosic components. In general, the supernatants obtained after alkali-treatment of rape seed as well as pea samples were more or less coloured, indicating that the extraction under N_2 was insufficient to avoid oxidative degradation completely. Neutralization with acetic acid reduced the colour slightly and the spectrophotometric measurements were performed on the pH adjusted samples. However, this procedure exclude contribution from UV absorbent compounds associated to hemicellulose A. The presence of

TABLE 9

Content of cinnamic acid derivatives in pectic material, hemicelluloses, and cellulose (DM basis) from pea IDF and SDF. The maximum value within the individual DF sources are shown in italics bold. The figures in percentage express the amount of UV absorbent material retained after dialysis of the supernatants. b.d. = before dialysis; a.d. = after dialysis; n.d. = non-dialysed. Missing observations are marked by "–". Data are relative values, denoted mmol, based on use of $\varepsilon = 15000\ M^{-1}$ for cinnamic acid in $E = \varepsilon \cdot c \cdot l$

Sample		Cinnamic acid content, μmol/g isolated DF							
		Pectic material			Hemicelluloses			Cellulose	SUM
		b.d.[1]	a.d.[2]	%	b.d.[1]	a.d.	%	n.d.[1]	
SOL	IDF	5.6	2.2	38.4	*31.1*	5.2	16.9	2.2	38.9
	SDF	*5.8*	5.1	86.6	1.6	0.3	20.0	0.6	8.0
KEL	IDF	2.6	1.1	41.7	*29.8*	3.8	12.9	4.0	36.4
	SDF	*3.7*	2.0	54.4	0.3	0.1	51.9	0.1	4.0
BOH	IDF	–	0.6	–	*20.2*	3.3	16.3	2.7	23.5*
	SDF	–	*1.6*	–	0.3	0.1	33.3	0.0	1.9*
BOH1	IDF	2.7	1.0	36.4	*24.5*	4.4	17.8	3.2	30.3
	SDF	*3.6*	1.8	50.0	0.3	0.1	40.9	0.0	4.0
BOH2	IDF	2.3	0.7	28.2	*29.8*	6.1	20.4	2.3	34.5
	SDF	*4.4*	2.1	48.7	1.1	0.2	13.0	0.1	5.6
PHU	IDF	–	0.6	–	*24.5*	3.2	13.0	5.5	30.6*
	SDF	–	*3.5*	–	1.1	1.9	–	0.1	4.7*

cinnamic acid derivatives in isolated DF has been confirmed by use of HPCE-MECC (Bjergegaard et al., 1992, 1997b) and combined with the recently developed SFE technique (Buskov et al., 1997) this gives an efficient tool for determination of such DF associated compounds.

UV-VIS-spectroscopy resulted in useful information on the amount of DF associated material of non-carbohydrate origin in the fractions. Generally, the IDF fraction showed the highest absorption at 280 nm and 320 nm, and the level was considerably higher for rape seed than for peas. The data showed, that especially the HEM fraction contained proteins and/or benzoic and cinnamic acid derivatives. However, this information has also been followed up by more specific methods in order to get a determination of the actual type and amount of compounds. SDS-PAGE and IEF of proteins and HPCE for proteins and LMW compounds associated to DF have been found to be suitable methods in this respect, as already demonstrated for phenolic carboxylic acids (Bjergegaard et al., 1992, 1997b), LMW carbohydrates (Arentoft et al., 1992; Frias et al., 1996) and various types of other LMW compounds as well as for proteins (Bjergegaard et al., 1997b).

TABLE 10

Content of cinnamic acid derivatives in pectic material, hemicelluloses and cellulose (DM basis) from rapeseed IDF and SDF. Comments and footnotes as in Table 9

Sample		Cinnamic acid content, μmol/g isolated DF							
		Pectic material			Hemicelluloses			Cellulose	SUM
		b.d.[1]	a.d.[2]	%	b.d.[1]	a.d.	%	n.d.[1]	
MRS	IDF	15.0	2.3	15.1	*71.2*	12.2	17.2	2.5	88.8
	SDF	*4.9*	3.7	75.4	1.0	0.3	26.4	1.0	6.9
MRSW	IDF	8.7	1.3	14.6	*170.4*	30.0	17.6	1.7	180.9
	SDF	*5.2*	4.5	87.0	2.5	0.7	28.7	0.6	8.3
NRS	IDF	14.5	2.5	17.4	*160.4*	37.9	23.6	3.3	178.2
	SDF	*10.9*	8.6	79.2	0.6	0.4	64.2	1.2	12.7
PRM	IDF	10.3	3.8	37.3	*100.0*	10.9	10.9	2.0	112.3
	SDF	*9.0*	7.7	85.3	3.7	0.4	10.1	0.8	13.4
NRH	IDF	6.7	1.5	22.9	*381.5*	87.2	22.9	3.1	391.3
	SDF	*8.6*	6.1	71.5	3.0	0.9	31.1	1.4	13.0
LIB	IDF	16.2	2.1	13.2	*96.0*	29.9	31.2	2.2	114.3
	SDF	*5.8*	4.6	78.9	5.3	0.8	15.7	0.7	11.8
LIB1	IDF	14.9	1.8	12.1	*123.5*	37.7	30.5	2.0	140.4
	SDF	*7.2*	6.2	85.9	6.6	0.7	10.0	0.7	14.6
BIN1	IDF	10.3	1.9	18.6	*138.1*	37.3	27.0	2.4	150.8
	SDF	*9.8*	8.8	89.6	3.7	0.8	22.1	0.7	14.2

Chromatographic techniques with these new developments (Bjergegaard et al., 1997b) are well suited for analysis of DF including determination of the NSP monosaccharides composition as a first step, but also in respect to oligo- and polysaccharides as well as non-traditional DF components as LMW phenolics and oligosaccharides. Up till now, GLC and HPLC have been the techniques preferred for monosaccharide analysis, however, HPCE provides a good alternative. The enzymatic gravimetric methods for determining IDF, SDF and TDF are good starting points for chromatographic investigations and further fractionation studies, but are also valuable themselves.

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REFERENCES

AOAC, 1965. Association of Official Analytical Chemists. Official Methods of Analysis. Washington D.C., p. 139

Albersheim P., Nevins D.J., English P.D., Karr A., 1967. A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. Carbohydr. Res. 5, 340-345

Arentoft A.M., Sørensen H., 1992. α-Galactosides and dietary fibres in relation to pea quality; Methods of oligosaccharide analysis. 1st European Conference on Grain Legumes, Angers (France), pp. 457-459

Asp N.G., 1990. Delimitation problems in definition and analysis of dietary fiber. In: I. Furda, C.J. Brine (Editors). New developments in dietary fiber – physiological, physicochemical, and analytical aspects. Plenum Press, New York/London, pp. 227-236

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

Asp N.G., Björck I., Holm J., Nyman M., Siljeström M., 1987. Enzyme resistant starch fractions and dietary fibre. Scand. J. Gastroenterol. 22, 29-32

Asp N.G., Furda I., Schweizer T.F., Prosky L., 1988. Dietary fiber definition and analysis. Amer. J. Clin. Nutr. 48, 688-690

Bagger C., Sørensen H. Sørensen J.C., 1996. High quality oil, protein and bioactive products, Food and non-food purpose based on biorefining of cruciferous oilseed crops. Congress on plant protein for European Crops. Food and non-food application, Food and non-food application. Nantes (France)

Bjerg B., Olsen O., Rasmussen K.W., Sørensen H., 1984. New principles of ion-exchange techniques suitable to sample preparation and group separation of natural products prior to liquid chromatography. J. Liquid Chromatogr. 7, 691-707

Bjergegaard C., Michaelsen S., Sørensen H., 1992. Determination of phenolic carboxylic acids by micellar electrokinetic capillary chromatography. J. Chromatogr. A, 608, 403-411

Bjergegaard C., Ochodzki P., Rakowska M., Sørensen H., 1995. Studies on enzyme based fractionation, chemical composition and biological effects of dietary fibres in rape seed (*Brassica napus* L.). I. Chemical composition of seeds and characteristics of soluble and insoluble dietary fibres of spring and winter type varieties of double low oilseed rape. J. Anim. Feed Sci. 4, 127-138

Bjergegaard C., Sørensen H., Sørensen S., 1997a. Dietary fibres – important parts of high quality foods and feeds. J. Anim. Feed Sci. 6, 145-161

Bjergegaard C., Michaelsen S., Sørensen, H., Sørensen, S., 1997b. Chromatography and Capillary Electrophoresis in Food Analysis. The Royal Society of Chemistry, Cambridge, UK (In press)

Brett C., Waldron K., 1990. Cell wall structure and the skeletal functions of the wall. In: Physiology and biochemistry of plant cell walls. Unwin Hyman, London, 194 pp.

Buskov S., Sørensen H., Sørensen J.C., Sørensen S., 1997. Extraction of oil from plant material with Supercritical carbon dioxide. Polish J. Food Nutr. Sci. (In press)

Brett C., Waldron K., 1990. Physiology and biochemistry of plant cell walls. Union Hyman, London, 194 pp.

Dreher M.L., 1987. Conventional and unconventional dietary fiber components. In: Handbook of dietary fiber. An applied approach. Marcel Dekker Inc., New York/Basel, pp. 17-49

Englyst H.N., Cummings J.H., 1988. Improved method for measurement of dietary fiber as non-starch polysaccharides in plant foods. J. Assoc. Off. Anal. Chem. 71, 808-814

Frias J., Price K.R., Fenwick G.R., Hedley C.L., Sørensen H., Vidal-Valverde C., 1996. Improved method for the analysis of α-galactosides in pea seeds by capillary zone electrophoresis. Comparison with high performance liquid chromatography triple-pulsed amperametric detection. J. Chromatogr. 719, 213-219

Fry S.C., 1988. Wall polymers: extraction and fractionation. In: M. Wilkins (Editor). The growing plant cell wall: chemical and metabolic analysis. Longman Scientific and Technical, pp. 49-101

Furda I., 1981. Simultaneous analysis of soluble and insoluble dietary fiber. In: W.P.T. James, O. Theander (Editors). The analysis of dietary fiber in food. Marcel Dekker Inc., New York/Basel, pp. 163-172

Jensen S.K., Olsen H.S., Sørensen H., 1990. Aqueous enzymatic processing of rape seed for production of high quality products. In: F. Shahidi (Editor). Rapeseed/Canola: Production, chemistry, nutrition and processing technology. Van Nostrand Reinhold Publisher, New York, pp. 331-343

Lee S.C., Prosky L., DeVries J.W., 1992. Determination of total, soluble and insoluble dietary fiber in foods. Enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study. J. Assoc. Off. Anal. Chem. Int. 75, 395-416

Olsen H.S., 1988. Aqueous enzymatic extraction of rape seed oil. Workshop on Agricultural Refineries. Bornholm, Denmark, 10 pp.

Prosky L., Asp N.G., Furda I., DeVries J.W., Schweizer T.F., Harland B.F., 1984. Determination of total dietary fiber in foods, food products and total diets: Interlaboratory study. J. Assoc. Off. Anal. Chem. 67, 1044-1052

Prosky L., Asp N.G., Furda I., DeVries J.W., Schweizer T.F., Harland B.F., 1985. Determination of total dietary fiber in foods and food products: Collaborative study. J. Assoc. Off. Anal. Chem. 68, 677-679

Prosky L., Asp N.G., Schweizer T.F., DeVries J.W., Furda I., 1988. Determination of insoluble, soluble and total dietary fiber in foods and food products: Interlaboratory study. J. Assoc. Off. Anal. Chem. 71,1017-1023

Prosky L., DeVries J.W., 1992a. Development of the association of official analytical chemists method for total, soluble and insoluble dietary fiber. In: Controlling dietary fiber in food products. Van Nostrand Reinhold, New York, pp. 47-90

Prosky L., DeVries J.W., 1992b. Regulations and marketing. In: Controlling dietary fiber in food products. Van Nostrand Reinhold, New York, pp. 91-122

Schweizer T.F., Würsch P., 1979. Analysis of dietary fibre. J. Sci. Food Agric. 30, 613-619

Selvendran R.R., Stevens B.J.H., O'Neill M.A., 1985. Developments in the isolation and analysis of cell walls from edible plants. In: C.T. Brett, J.R. Hillman (Editors). Biochemistry of plant cell walls. Cambridge University Press, Cambridge/London/New York/New Rochelle/Melbourne/Sydney, pp. 39-78

Southgate D.A.T., 1991. The measurement of unavailable carbohydrates: Structural polysaccharides and dietary fibre. In: Determination of food carbohydrates. Elsevier Applied Science, London/New York, pp. 73-92

Theander O., 1983. Advances in the chemical characterization and analytical determination of dietary fibre components. In: G.G. Birch, K.J. Parker (Editors). Dietary fibre. Applied Science Publisher, London/New York, pp. 77-93

Van Soest P.J., 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Off. Anal. Chem. 46, 829-835

Van Soest, P.J., Wine R.H. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. J. Assoc. Off. Anal. Chem. 50, 50-55

STRESZCZENIE

Porównanie włókna pokarmowego i związków towarzyszących DF w rzepaku i produktach jego frakcjonowania oraz DF grochu

Włókno pokarmowe (DF) – jego skład chemiczny, budowa, właściwości fizyko-chemiczne i działanie fizjologiczne są obecnie przedmiotem rosnącego zainteresowania. Postęp w tych badaniach utrudniony jest przez brak uzgodnionej definicji pojęcia DF. W przedstawionych badaniach zastosowano fizjologicznie uzasadnioną metodę oznaczania ogólnej zawartości DF (TDF) jako sumy DF nierozpuszczalnego (IDF) i rozpuszczalnego (SDF) do porównania DF różnych odmian oraz frakcji rzepaku i DF grochu.

Przeprowadzono szeroką charakterystykę DF rzepaku i grochu obejmującą w pierwszym etapie oznaczenie różnymi metodami składu obojętnych cukrów prostych. Ilość cukrów, zwłaszcza glukozy, zależała w dużym stopniu od metody hydrolizy wielocukrów. Skład frakcji wielocukrów różnił się w zależności od rodzaju materiału roślinnego. Różnorodność tę potwierdziły wyniki ekstrakcji i rozdziału DF pozwalające na wyodrębnienie czterech grup związków: pektyn, hemicelulozy, celulozy i ligniny. Spektroskopia UV-VIS ekstraktów tych grup wykazała obecność związków nie będących węglowodanami. We frakcji hemicelulozy grochu, a zwłaszcza rzepaku, stwierdzono największą zawartość białek i fenoli, prawdopodobnie silnie adsorbowanych lub związanych kowalencyjnie. Część białkową frakcji DF analizowano następnie metodami biochemicznymi: chromatografii powinowactwa, elektroforezy z zastosowaniem SDS i IEF. W DF rzepaku stwierdzono obecność myrozynazy. Również groch zawierał białko związane z DF, jednak jego zawartość była znacznie mniejsza niż w DF rzepaku. Opracowano metody wysoko wydajnej elektroforezy kapilarnej do oznaczania związków fenolowych jako składnika DF. Zastosowanie tych metod do analizy DF i jego frakcji pozwoliło na stwierdzenie obecności znacznych ilości białka i różnych związków o niskiej masie cząsteczkowej, zwłaszcza fenoli, jako składników powiązanych z DF.