CARBOHYDRATES IN RAPESEED AND TURNIP RAPESEED MEALS

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Except for the glucosinolates, the carbohydrates in rapeseed and turnip rapeseed are so far uncompletely studied. The characterisation of the low-molecular as well as the polysaccharide components is important to the fodder and food processor, the plant breeder and the nutritionist. Our investigations are in particular connected with research and development in Sweden during the last years, concerning a process to produce rapeseed protein concentrate for human consumption.

Mono- and oligosaccharides, sugar alcohols and cyclitols

Very recently, SIDDIQUI et al. (1973) reported studies on low-molecular carbohydrates from dehulled and fat-free meal of Brassica campestris using chromatography on a carbon - Celite column. In independent studies we have determined the main low-molecular carbohydrates (soluble in 80 % aqueous ethanol) in a series of rapeseed and turnip rapeseed meals (dehulled, heat-treated and defatted). Between 29-36 % of the dry weight of the meal was thus extracted. After extraction with chloroform (removing small amounts of lipids left) and treatment with cation- and anion exchange resins (to remove glucosinolates, amino acids and other ionic components), the neutral, water-soluble components were studied.

Figure 1 shows an example of fractionation of low-molecular carbohydrates from rapeseed meal by gel filtration on Sephadex G 15, using eluation with water. The fractions were studied by paper- and gas-liquid chromatography (GC) after transformation of the components into their volatile trimethylsilyl (TMS) derivatives or acetates. Galactinol in fraction V is a minor component, composed of one mole each of galactose and the cyclitol myo-inositol, and galactitol in fraction VII the sugar alcohol, which corresponds to galactose.

GC was used for identification of various components by comparison of their retention times with those of authentic samples and sometimes, to get further support for a structure, in combination with mass spectrometry. GC is a rapid, accurate technique (after a proper determination of response factors) for quantitative determination of the various components. This can be done (except for the very minor ones) directly on the mixture of neutral components, without subfractionation by gel filtration. For the determination of monomeric carbohydrates (and sucrose) as TMS derivatives a 3 % OV-1 column is very suitable; for oligosaccharides a 3 % OV-17 column is to be preferred.

Figure 2 illustrates GC-separation of oligosaccharides with myo-inositol as an internal standard. The areas of the peaks are proportional to the amounts of the respective compounds, and corrections are then made using

Figure 1:

Fractionation of low-molecular carbohydrates from rapeseed meal (Brassica napus cv. Victor) on Sephadex G15.

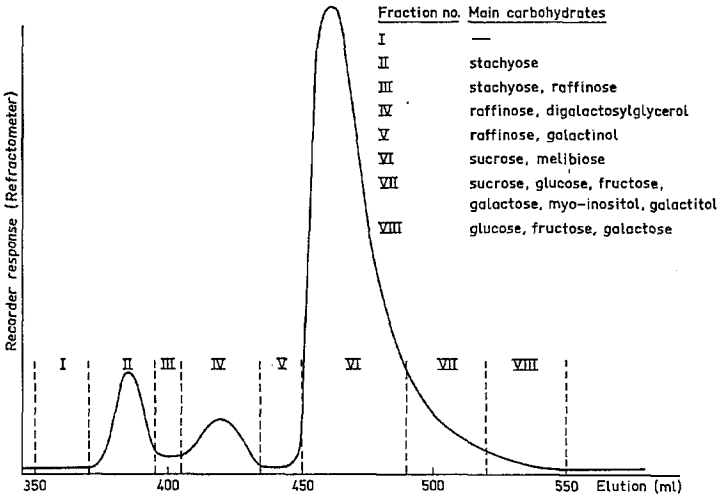
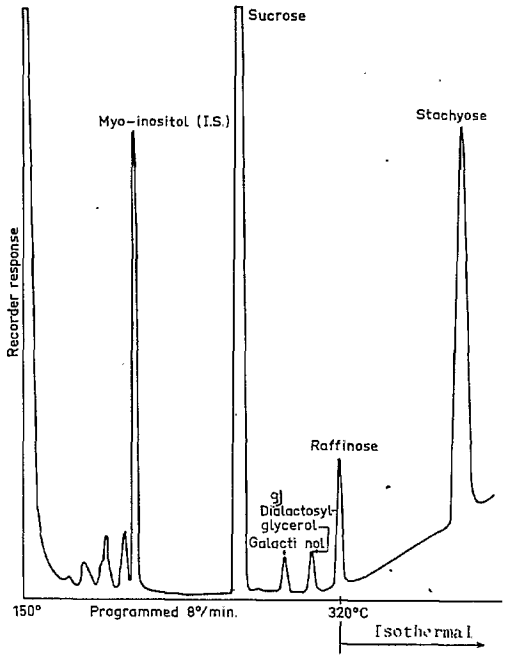


Figure 2:

Oligosaccharides in rapeseed meal determined with GLC (3% OV-17) as TMS-derivatives

Isothermal



individual response factors.

In Table 1, the amounts of the major low-molecular carbohydrates in six different rapeseed and turnip rapeseed meals are presented. The predominant components are sucrose and stachyose, and the total amount of the five saccharides accounts for 10-12 % of the dry weight of defatted meal.

Table 1: Low-molecular carbohydrates [a]

	Fructose	Glucose	Sucrose	Raffinose	Stachyose
Brassica napus					
Winter type,					
cv. Victor	0.10	0.10	6.51	0.31	1.43
cv. Sinus	0.17	0.17	8.26	0.30	2.29
Summer type,					
cv. Gulle	0.27	0.22	7.48	0.29	3.04
cv. Bronowski-type, SV 71/6B	0.17	0.24	7.10	0.33	2.47
Brassica campestris					
Winter type,					
cv. Duro	0.51	0.40	7.49	0.31	2.39
Summer type,					
cv. Bele	0.24	0.32	6.77	0.34	2.43

[a] given as % of dry defatted meal

The sucrose and stachyose values for all our six samples are higher than the values found by SIDDIQUI et al. (1973) in their investigation, i.e. 2.26 and 1.52 respectively. Seasonal and other variations may perhaps explain these differences.

Stachyose and raffinose are frequently claimed to have intestinal gas-forming properties. In the technical protein concentrate process, however, the low-molecular saccharides, as well as the glucosinolates are removed by water extraction.

Glucosinolates

The structures and distribution of glucosinolates in various Brassica seeds are well studied, and several excellent reviews have been published. In the present investigation, we developed a method for rapid determination of total glucosinolate content (using an average molecular weight for the actual glucosinolates of 380 as an approximation). The extraction residue from ultrasonic treatment of a sample with 80 % aqueous ethanol at

room temperature was evaporated and then hydrolysed enzymatically with myrosinase and the glucose formed was determined by GC (Fig. 3). The increase of glucose from the trace amount present before hydrolysis is proportional to the glucosinolate content. The results have been in good agreement with those obtained by other more circumstantial methods. A method for individual quantitative determination of different glucosinolates, based on GC of the glucosinolate-TMS derivatives has recently been developed by UNDERHILL and KIRKELAND (1971) and by the Swedish Karlshamn group. Fig. 4 shows a typical gas chromatogram with the four main glucosinolates and with sinigrin as an internal standard. These TMS

Figure 3:

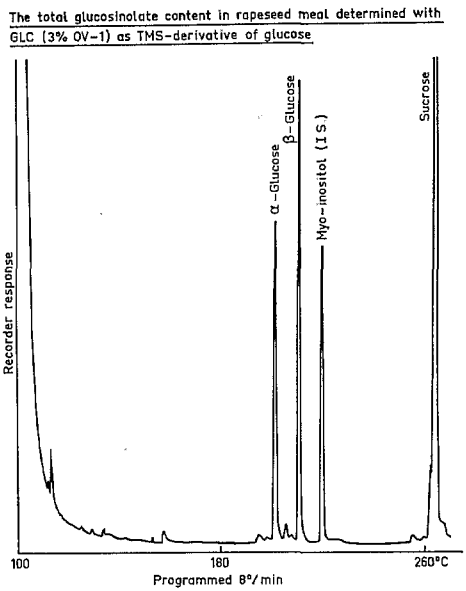
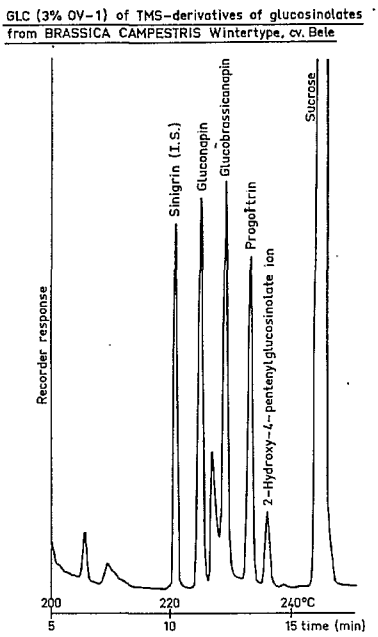


Figure 4:



derivatives fit in very nicely between those of the mono- and oligosaccharides. Table 2 presents the total glucosinolate content of the same six rapeseed and turnip rapeseed samples, which also have been analysed for sugars (Table 1). One can notice the low glucosinolate content of the Bronowski sample. The GC methods for glucosinolates and sugars have turned out to be suitable for analysis of technical water extractives in the protein concentrate process.

To support for identification of different glucosinolates, we have studied the mass spectra of the TMS derivatives using the GC-mass spectrometry combination, which is a modern, very valuable tool in studies of natural products.

Table 2: The total glucosinolate content in rapeseed and turnip rapeseed

	Glucosinolate content [a]
Brassica napus	
Winter type,	
cv. Victor	7,4
cv. Sinus	4,4
Summer type,	
cv. Gulle	6,7
cv. Bronowski-type SV 71/6B	2,5
Brassica campestris	
Winter type,	
cv. Duro	4,7
Summer type,	
cv. Bele	4,9

[a] Given as % of dry defatted meal

Figure 5:

Mass spectrum of the TMS-derivative of Progoitrin (2-Hydroxy-3-Butenylglucosinolate ion)

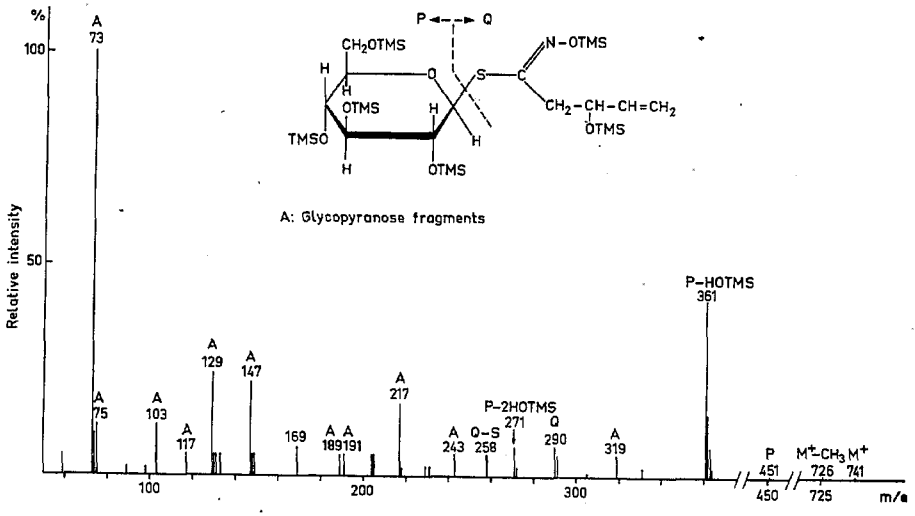
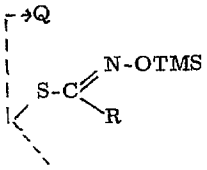


Fig. 5 shows the mass spectrum of the TMS derivative of progoitrin as an example. The molecular ion (M^+; 741) as well as M^+-CH_3 (726) are seen. A series of peaks, which are characteristic for TMS derivatives of glucosides in general, are marked with A. The predominant peak at m/e 361, corresponding to P-HOTMS, and at m/e 271 (P-2 HOTMS) were more intense than for a simple methyl glucoside. The peaks at m/e 290, corresponding to Q, and at m/e 258 (Q-S) are characteristic of progoitrin. Table 3 shows the relative intensities of some characteristic peaks originating from the Q part of four glucosinolates, and illustrates how useful the method is.

Table 3: Relative intensities (m/e 147 = 100 %) of some peaks in mass spectra of TMS-derivatives of glucosinolates

Glucosinolate	R	Q	Q-S	Q-(S+HOTMS)	Q-(S+HOTMS+HCN)
Sinigrin	$-CH_2-CH=CH_2$	-	34	-	-
Gluconapin	$-CH_2-CH_2-CH=CH_2$	20	15	45	-
Glucobrassicanapin	$-CH_2-CH_2-CH_2-CH=CH_2$	-	-	26	41
Progoitrin	$-CH_2-CH(OTMS)-CH=CH_2$	32	22	-	-

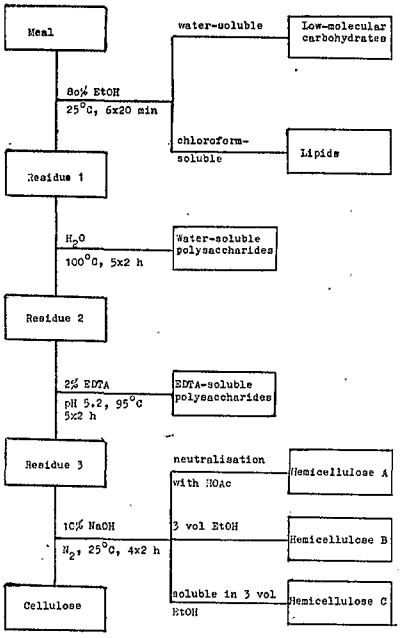


Polysaccharides

As seen in Fig. 6, the residues after extraction with 80 % ethanol were subsequently extracted with water, EDTA-solution (generally used for extraction of pectin) and 10 % aqueous sodium hydroxide (extracting the so-called hemicellulose). The hemicelluloses were sub-fractionated into (A) those precipitated with acetic acid, (B) those precipitated with ethanol from the mother liquor of (A), and (C) those soluble in aqueous ethanol. A large sample of defatted meal from Brassica napus cv. Sinus was fractionated in this way. The yields of these fractions, their content of neutral carbohydrates (the uronic acid determination is not finished) and the relative composition of each fraction (determinated by GC on hydrolysates) are given in Table 4. The carbohydrates in these fractions are present as polysaccharides and the amount of neutral constituents in these represent around 11 % of dry defatted meal. When also the acidic carbohydrate constituents can be included the total carbohydrate content will probably amount to less than 20 %. The main part in the defatted meal after extraction with aqueous ethanol is protein. Also the remaining "cellulose"-frac-

Figure 6:

Extraction scheme of carbohydrates in rapeseed meal (dehulled, heat treated, oil extracted and milled).



tion contains a considerable amount of protein, as shown by an X-ray study (A. von HOFSTEN). The cellulose showed a typical fibre structure. Arabinose is a major component in the non-cellulosic polysaccharides; xylose and galactose are also important neutral components. The lignin content of the meal seems to be minor. We are at present engaged in fractionation and characterisation studies on various polysaccharides in these fractions. SIDDIQUI and WOOD previously (1971, 1972) isolated small amounts of an amyloid (consisting of D-glucose, D-galactose and D-xylose) and an arabinogalactan (consisting of L-arabinose, D-galactose and D-glucuronic acid) from the watersoluble polysaccharides from rapeseed (Brassica campestris) and discussed their general structural features.

We have sub-fractionated the water-soluble polysaccharides from Brassica napus cv. Sinus by precipitation with ethanol. The more ethanol soluble part was fractionated by gel filtration as shown in Fig. 7.

Peak A represents a mixture of at least two high-molecular polysaccharides, consisting mainly of arabinose and galactose. Peak B represents an

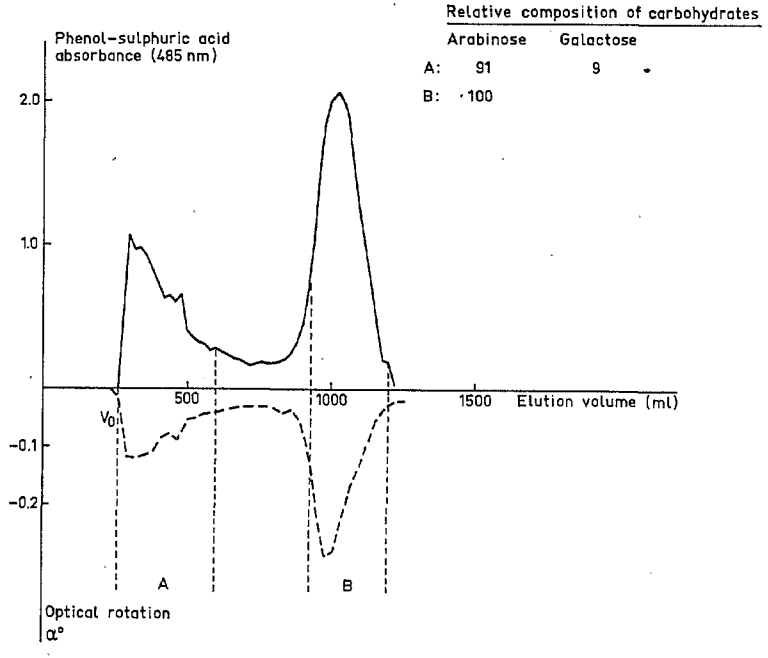
Table 4: Polysaccharide fractions from Brassica napus Winter type cv. Sinus (50 g)

Carbohydrate fractions	Yield[a]	Content of neutral carbohydrates[b]	Relative composition of neutral components[c]						
			Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose
Water-soluble polysaccharide	12	14	2	1	65	5	1	14	12
EDTA-soluble polysaccharide	18	18	4		61	13		15	7
Hemicellulose A	8	3	8	5	58	30			
Hemicellulose B	10	30	4		28	27	2	14	26
Hemicellulose C	10	-							
Cellulose	5	55			6	4		2	88

[a] Given as % of dry defatted meal
[b] Given as % of carbohydrate fraction
[c] Uronic acids not yet determined

Figure 7:

Purification of water-soluble arabinan on Sepharose 2 B



arabinan, containing only arabinose units. We are at present performing structural studies on this polysaccharide. The highly negative optical rotation ($[\alpha]_D$ -83°) of the polysaccharide, which after complete hydrolysis to arabinose turns into positive rotation, indicates furanosidically linked α -L-arabinose residues. GC analysis of fragments resulting from hydrolysis of the methylated polysaccharide shows equal amounts of 2,3,5-tri-O-methyl-L-arabinose, 2,3-di-O-methyl-L-arabinose and 2-O-methyl-L-arabinose, indicating that the polysaccharide is highly branched. NMR-studies in pyridine indicate three main types of arabinose units (three peaks for different anomeric protons were obtained). We hope soon to complete the structural elucidation, using partial degradation studies. The polysaccharide seems to be related but not identical to the previously (by REES and RICHARDSON, 1966) presented, possible structures for an arabinan in white mustard seeds.

The other polysaccharides in the water extract, which are more readily precipitated with ethanol, consist of arabinose, galactose, glucose and uronic acids.

The polysaccharides in the EDTA-extract seem to consist mainly of acidic pectins, as expected, but present fractionations using chromatography on

DEAE-Sephadex and Sepharose 2B columns also yield neutral polysaccharides with arabinose as the main constituent. In the near future, we are going to fractionate and characterise the hemicellulose polysaccharides.

References

1. HOFSTEN, A. v. (1970): In: Proceedings Intern. Conference on Rapeseed, St. Adèle, Quebec, Canada 1970, p. 70-85

2. REES and RICHARDSON (1966)

3. SIDDIQUI, I.R., P.J.WOOD and G.KHANZADA (1973): J. Sci. Food Agric. 24, 1427-1435

4. SIDDIQUI, I.R. and P.J.WOOD (1971, 1972)

5. UNDERHILL and KIRKLAND (1971): J. Chromatogr. 57, 47-54