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Content and Composition of Non-Starch Polysaccharides in Some Norwegian Plant Foods

R. Reistad

State Institute of Consumer Research,
Ringstabekkveien 105, N-1340 Bekkestua, Norway

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ABSTRACT

Analyses of non-starch polysaccharides have been carried out on plant material grown in Norway including potatoes, apples and some vegetables.

A method which determined both amount and type of polysaccharides was used.

INTRODUCTION

Based on the increasing interest in dietary fibre (DF) in recent years, it was considered important to know the content of DF in various plant materials used for human consumption. It has emerged that the various methods of analysis often give different results, due to the complex nature of the polymers involved. Much of the analytical work has been carried out on cereals, because of their potential as a rich source of DF. However, various vegetables also contain a substantial amount of DF on a dry weight basis.

The aim of this investigation was to derive information on DF in various plant materials other than cereals, which can be grown in a cool/temperate climate, are widely used for human consumption, have good storing properties and are relatively inexpensive. Samples of potatoes, apples and some vegetables were chosen for analysis.

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Information on amount, as well as type, of DF was considered of interest.

MATERIALS

All reagents were analytical grade. Monosaccharides: Sigma Chemical Co. Amyloglucosidase from *Aspergillus niger*, EC 3.2.1.3 and pullulanase from *Aerobacter aerogenes*, EC 3.2.1.41: Boehringer Mannheim GmbH. α-Amylase from porcine pancreas, A4268, EC 3.2.1.1: Sigma Chemical Co. Pyridine and acetic acid anhydride were distilled prior to use, pyridine over KOH pellets.

METHODS

DF was analysed essentially according to the Englyst (1981) procedure, based on the method of Southgate *et al.* (1978). Free sugar and starch determinations were omitted.

Preparation of samples

Samples were prepared within 24 h of purchase from Gartnerhallen A/L, the major Norwegian potato, vegetable and fruit distributor. The following material, all grown in Norway, was collected: cabbage (variety Golden Acre), cauliflower (Bravo), rutabagas (Gry), carrots (Nantes), onions (Hygro), tomatoes (Virosa), potatoes (Kerrs Pink) and apples (James Grieve). Cabbage and cauliflower were collected in July, rutabagas, onions, carrots and tomatoes in August, and potatoes and apples in September. All material was used without processing. Of each variety, samples were collected from two producers and prepared for analysis together. This was done to minimise possible effects of cultivation and handling prior to preparation of samples for DF content.

The parts of the various plant materials normally used for human consumption were prepared for analysis. Rutabagas and potatoes were peeled, carrots were scraped, the cabbage stem was removed and from the onions the outer dry layers were removed. For apples, the flesh with skin was used. Tomatoes were used as purchased.

All material was diced (approx. 3 mm^3), frozen at −25 °C, freeze dried

and milled in a grain mill (Casella & Co., London) fitted with a 0·5 mm screen. Some of the material (rutabagas, tomatoes and apples) was difficult to run through the mill and was ground in a mortar. The powdered material was stored in small, tightly capped glass jars in desiccators at 4 °C above blue silica gel.

Due to the hygroscopic nature of some of the freeze-dried material, moisture determinations were carried out simultaneously with the DF analyses (Matthee & Appledorf, 1978).

Uronic acids

Uronic acids were determined as galacturonic acid by the harmine method of Wardi *et al.* (1974), with corrections for the presence of hexoses (Englyst, 1981).

Cellulose

Cellulose was measured as glucose according to the methods of Roe (1955).

Gas–liquid chromatography

Gas–liquid chromatography was performed on a Hewlett-Packard 5940A gas chromatograph equipped with flame ionization detector, using glass columns (2 mm inside diameter × 6 ft) with 3 % neopentyl glycol succinate on Chromosorb WAW, 100–120 mesh. Operating conditions: column temperature programming from 180 to 220 °C, at 2 °/min with a final 4·5 min hold at 220 °C, injection port, 225 °C, FID 250 °C, and nitrogen carrier gas at a flow rate of 27 ml/min.

The neutral monosaccharides were analysed as their aldonitrilacetates (Mergenthaler & Scherz, 1976). 1,2,3,5-4,6-hexahydroxycyclohexane and 1,2,3,4-butanetetrol were used as internal standards.

Sample analysis conditions

The main steps of the method are removal of starch, separation of soluble and insoluble DF and determination of the monosaccharides in the two fractions.

The analytical procedure is outlined in Fig. 1. Sample size was 200 mg, with 20 ml of sodium acetate buffer. For starch degradation either 4·2

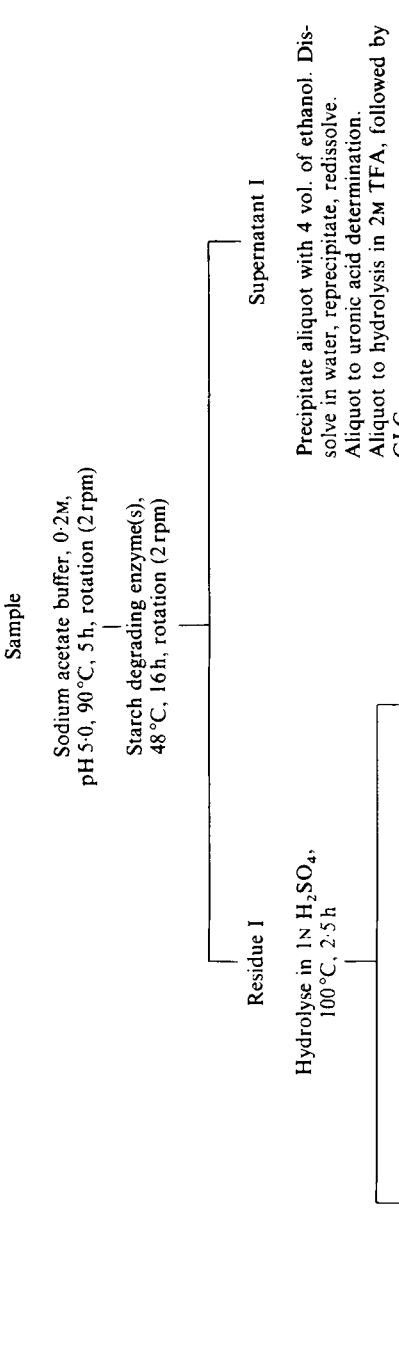

Fig. 1. Outline of analytical procedure.

units of amyloglucosidase or 600 units of α-amylase and 1 unit of pullulanase (Englyst *et al.*, 1982) were used. After incubation the sample was centrifuged, and aliquots of the supernatant precipitated with ethanol. Centrifugation after ethanol precipitation was carried out at $10\,000 \times g$ for 10 min at +4 °C, the other centrifugations being at $2000 \times g$ for 20 min at room temperature.

Liquids were evaporated on a rotary evaporator connected with a vacuum pump, and a bath temperature of 40 °C.

Treatment of Supernatant I (Fig. 1)

To a 2·5 ml aliquot in a corex tube was added 10 ml of ethanol. After thorough mixing the tube was left for 30 min before centrifugation. The supernatant was discarded and the precipitate dissolved in 2·5 ml of water and reprecipitated with ethanol. After dissolving in water, suitable aliquots were used for uronic acid determination as well as for hydrolysis in 2M trifluoroacetic acid (TFA) for 1 h at 120 °C after addition of internal standards. The hydrolysate was subjected to GLC after derivatisation.

Treatment of Residue I (Fig. 1)

After washing and the addition of internal standards, the residue was hydrolysed in 1N H_2SO_4 for 2·5 h at 100 °C, and filtered through a G4 glass filter crucible fitted with a glassfibre filter (GF/D) to aid filtration. Suitable aliquots of the filtrate (Filtrate I) were used for uronic acid determination. Aliquots were also neutralised with $BaCO_3$ and the neutral monosaccharides subjected to GLC after derivatisation.

The residue on the filter (Residue II) was washed extensively with water, acetone, and then air dried. To the dry residue in the crucible, placed in a small beaker, was added 15 ml of cold (+4 °C) 72 % H_2SO_4. The crucible was left at this temperature for 24 to 48 h with intermittent stirring to solubilise the cellulose. After filtration and dilution to 100 ml, cellulose was determined in the filtrate as glucose (Roe, 1955).

RESULTS AND DISCUSSION

Due to the relatively small sample size and the low content of non-carbohydrate residue (< 4 % of dry weight), this could not be accurately

determined gravimetrically and was omitted. The term 'non-starch polysaccharides', instead of DF, is therefore used in the Tables.

The harmine reagent used for the determination of uronic acid content (Wardi *et al.*, 1974) has the disadvantage of also reacting with hexoses, which has to be corrected for. To measure uronic acid content in the sulfuric acid hydrolysate of the water-insoluble non-cellulosic polysaccharides, an additional blank was prepared for each sample. In this blank the harmine reagent was omitted. The blank was necessary because a yellow–brown colour which absorbed at 530 nm was formed during the heat treatment, and had to be corrected for (Anderson & Clydesdale, 1980).

It has been reported that some preparations of amyloglucosidase also attacked β-glucans (Englyst *et al.*, 1982). As this enzyme was initially used in our studies, a comparison with α-amylase and pullulanase was carried out. However, on our material the results were quite similar (Table 1).

According to Englyst *et al.* (1982) the earlier method (Englyst, 1981), as also used by us, suffers from an incomplete removal of starch and a smaller proportion of non-starch polysaccharides as soluble polysaccharides due to extraction at pH 5 instead of pH 7.

Our results do not indicate an incomplete removal of starch, as the glucose content in the hydrolysate of the insoluble fraction is very low (see Table 4, p. 55).

With regard to an increase in the amount of polysaccharide extracted at pH 7, it is well known that the solubility depends upon the extraction conditions. Consequently, it may be difficult to define the most 'physiological' or 'correct' conditions for the extraction of soluble polysaccharides. We have therefore continued to use the extraction procedure, as outlined by Englyst (1981), until a common extraction procedure is agreed upon.

In the same procedure, the soluble carbohydrate fraction is hydrolysed with trifluoroacetic acid (TFA) and the insoluble fraction with H_2SO_4. In the Englyst *et al.* (1982) procedure, only H_2SO_4 is used.

In our work hydrolysis of the water-soluble polysaccharide fraction with TFA gave higher and more reproducible results for rhamnose which, in some samples, was completely absent after hydrolysis with H_2SO_4 (Table 2). The other monosaccharides did not differ much, whether hydrolysed with TFA or H_2SO_4. The latter was used for hydrolysis of the water-insoluble carbohydrate fraction.

Selvendran *et al.* (1979) used a common correction factor for rhamnose

TABLE 1

Neutral Constituents of Total Non-Cellulosic Polysaccharides After Treatment With Starch-Degrading Enzymes (Weight %)

Sample	*Starch-degrading enzymes*	*Neutr.*	*Rha.*	*Fuc.*	*Ara.*	*Xyl.*	*Man.*	*Gal.*	*Glu.*
Cabbage	Amyloglucosidase	5·2	0·2	0·1	2·1	0·7	0·1	1·8	0·2
	α-Amylase + pullulanase	4·9	0·2	0·1	2·0	0·6	0·1	1·7	0·2
Cauliflower	Amyloglucosidase	6·8	0·3	0·1	2·8	0·9	0·1	2·3	0·3
	α-Amylase + pullulanase	7·4	0·3	0·2	3·1	0·9	0·2	2·5	0·2
Rutabagas	Amyloglucosidase	5·2	0·3	<0·05	2·1	0·6	<0·05	2·0	0·2
	α-Amylase + pullulanase	5·3	0·3	0·1	2·1	0·5	0·1	2·0	0·2
Carrots	Amyloglucosidase	5·9	0·3		2·0	0·2	<0·05	3·3	0·1
	α-Amylase + pullulanase	6·2	0·3		2·0	0·2	0·1	3·4	0·2
Onions	Amyloglucosidase	3·4	0·1	<0·05	0·2	0·3	<0·05	2·7	0·1
	α-Amylase + pullulanase	3·5	0·1	<0·05	0·2	0·3	<0·05	2·8	0·1
Tomatoes	Amyloglucosidase	2·3	0·1	—	0·6	0·5	0·2	0·8	0·1
	α-Amylase + pullulanase	2·3	0·1	—	0·6	0·4	0·3	0·8	0·1
Potatoes	Amyloglucosidase	1·6	—	—	0·2	0·1	—	1·1	0·2
	α-Amylase + pullulanase	1·3	—	—	0·2	0·1	—	1·0	<0·05
Apples	Amyloglucosidase	4·0	<0·05	0·1	1·7	0·5	<0·05	1·6	0·1
	α-Amylase + pullulanase	3·9	<0·05	0·1	1·7	0·5	<0·05	1·5	0·1

— = None detected.

Neutr. = Sum of neutral constituents.

Rha. = rhamnose; Fuc. = fucose; Ara. = arabinose; Xyl. = xylose; Man. = mannose; Gal. = galactose; Glu. = glucose.

TABLE 2

Constituents of Water-Soluble, Non-Starch Polysaccharides After Hydrolysis in Sulfuric Acid or Trifluoroacetic Acid (Weight %)

Sample	*Hydrolysis*	*Rha.*	*Fuc.*	*Ara.*	*Xyl.*	*Man.*	*Gal.*	*Glu.*
Cabbage	1N H_2SO_4	<0·05	—	1·0	<0·05	—	0·7	—
	2M TFA	0·1	—	0·9	<0·05	—	0·6	—
Cauliflower	1N H_2SO_4	—	—	2·0	0·1	—	1·0	—
	2M TFA	0·2	—	1·7	0·1	<0·05	1·0	
Rutabagas	1N H_2SO_4	0·1	—	1·6	—	—	1·2	—
	2M TFA	0·3	—	1·7	—	—	1·2	—
Carrots	1N H_2SO_4	0·1	—	1·3	<0·05	—	2·1	—
	2M TFA	0·2	—	1·2	<0·05	—	2·0	—
Onions	1N H_2SO_4	<0·05	—	0·1	—	—	1·7	—
	2M TFA	0·1	—	0·1	<0·05	—	1·7	—
Tomatoes	1N H_2SO_4	—	—	0·3	—	—	0·4	—
	2M TFA	0·1	—	0·3	<0·05	—	0·4	<0·05
Potatoes	1N H_2SO_4	—	—	0·1	—	—	0·4	—
	2M TFA	—	—	0·1	—	—	0·3	—
Apples	1N H_2SO_4	—	—	0·9	<0·05	—	0·6	—
	2M TFA	<0·05	—	0·8	<0·05	—	0·5	—

— = None detected.

See also footnotes to Table 1.

TABLE 3
Constituents of Water-Soluble, Non-Starch Polysaccharides, Calculated as Polymers (Weight %)

Sample		*Neutr.*	*Ur. a.*	*Rha.*	*Fuc.*	*Ara.*	*Xyl.*	*Man.*	*Gal.*	*Glu.*
Cabbage	(R)	1·6	3·4	0·1		0·9	<0·05		0·6	
	(E I)	3·4	9·6	0·4	*	1·6	0·1	tr.	1·0	0·3
	(E II)	5·97	7·56	0·85	0·10	3·14	0·19	0·05	1·26	0·38
	(T)	0·9	1·9	0·1		0·3	0·1		0·4	
Cauliflower	(R)	3·0	4·7	0·2		1·7	0·1	<0·05	1·0	
	(E I)	4·9	5·0	0·5	*	2·2	tr.	tr.	1·8	0·4
Rutabagas	(R)	3·0	6·0	0·3		1·5	<0·05		1·2	
Carrots	(R)	3·4	5·1	0·2		1·2	<0·05		2·0	
	(E I)	5·1	6·0	0·3	*	1·4	tr.	tr.	2·6	0·8
	(T)	0·9	2·0	0·1		0·3	tr.		0·4	0·1
Onions	(R)	1·9	3·0	0·1		0·1	<0·05		1·7	
	(E I)	4·0	3·6	0·2	*	0·2	tr.	tr.	3·4	0·2
Tomatoes	(R)	0·8	3·5	0·1		0·3	<0·05		0·4	<0·05
	(E I)	2·6	7·4	0·3	*	0·5	0·1	0·2	1·2	0·3
Potatoes	(R)	0·4	0·5			0·1			0·3	
	(E I)	2·1	1·5	0·1	*	0·4	tr.	0·1	0·7	0·8
	(E II)	1·74	1·02	0·13		0·26	0·01	tr.	1·25	0·09
Apples	(R)	1·3	2·8	<0·05		0·8	<0·05		0·5	
	(E I)	1·2	4·1		*	0·6	0·1	tr.	0·3	0·2
	(T)	0·5	2·3	tr.		0·3	tr.	tr.	0·1	0·1

*No information, whether not determined or below detection limit.
Unless otherwise indicated, absence of a figure means that none was detected.
(E I) = Englyst (1981); (E II) = Englyst *et al.* (1982); (T) = Theander & Åman (1979); (R) = Reistad (present study).
tr = trace; Neutr. = sum of neutral constituents; Ur. a. = uronic acids.
See also footnotes to Table 1.

to correct for a slow liberation of this monosaccharide in H_2SO_4. However, as our results showed a varied degradation pattern of rhamnose from sample to sample (Table 2), no common correction factor could be used. As hydrolysis in TFA seemed to eliminate the problem, this was not investigated further.

In our work, monosaccharides were converted to their aldonitrilacetates prior to GLC, while alditolacetates are more commonly used. A comparison of the two derivatisation procedures gave very similar results (Englyst, 1981). As we have found aldonitrilacetates satisfactory, we have continued to use these derivatives.

A comparison of our results with those of Englyst (1981), Englyst *et al.* (1982) and Theander & Åman (1979) is shown in Tables 3 to 6. Some of the figures of the other authors have been recalculated for comparison purposes, as they were originally presented as relative, rather than absolute, values (Theander & Åman, 1979), or as monomers, rather than polymers (Englyst, 1981).

Our results are generally lower than those of the other authors—a fact most clearly seen in Table 6. There are fairly large differences from method to method for most of the components (Tables 3 and 4), even for presumably identical methods (R, EI). For some of the components the differences are somewhat less when total amounts are compared (Table 5). However, the major part of the differences cannot be explained by different amounts of water-soluble material being extracted by the various procedures. It is more probable that the differences are caused by the nature of the samples.

Treatment of our samples prior to analysis is described in detail under 'Methods'. Peeling, removal of stems, etc., will undoubtedly influence the content of DF, as, most likely, also will variety, cultivation and growth conditions, length and conditions of storage, etc. To our knowledge this has not been systematically investigated.

As the material investigated is normally not consumed as dry material, it was, in addition, calculated on a fresh weight basis, as was also done by Theander & Åman (1979). The results of the two methods are close for cabbage and carrots, whilst those of apples are quite different. The latter results probably reflect a great difference between varieties.

In principle, there is not much difference between the methods of Theander & Åman (1979), Englyst (1981) and Englyst *et al.* (1982), but different solutions have been chosen for the isolation and determination of the constituents of the DF polymers.

TABLE 4
Constituents of Water-Insoluble, Non-Starch Polysaccharides, Calculated as Polymers (Weight %)

Sample		*Neutr.*[x]	*Ur. a.*	*Cellul.*	*Rha.*	*Fuc.*	*Ara.*	*Xyl.*	*Man.*	*Gal.*	*Glu.*[x]
Cabbage	(R)	3·3	1·9	6·3	0·1	0·1	1·1	0·6	0·1	1·1	0·2
	(E I)	4·1	ND	9·3	tr.	*	1·3	1·0	tr.	1·3	0·5
	(E II)	3·69	0·52	9·86	0·05	0·07	0·84	1·33	0·64	0·76	
	(T)	5·3	2·7	9·0	0·5	0·2	1·3	1·4	0·6	1·3	+
Cauliflower	(R)	4·4	1·5	7·1	0·1	0·2	1·4	0·8	0·2	1·5	0·2
	(E I)	4·6	ND	12·5	tr.	*	1·5	1·2	0·3	1·2	0·4
Rutabagas	(R)	2·3	0·9	6·6	<0·05	0·1	0·6	0·5	0·1	0·8	0·2
Carrots	(R)	2·8	0·9	5·3	0·1		0·8	0·2	0·1	1·4	0·2
	(E I)	3·3	ND	9·0	0·1	*	0·7	0·4	tr.	1·3	0·8
	(T)	6·0	5·5	9·1	0·6	tr.	1·7	1·0	0·8	1·9	+
Onions	(R)	1·6	0·4	3·5	<0·05	<0·05	0·1	0·3	<0·05	1·1	0·1
	(E I)	1·5	ND	4·0	tr.	*	0·2	0·3	0·1	0·5	0·4
Tomatoes	(R)	1·5	0·4	5·2			0·3	0·4	0·3	0·4	0·1
	(E I)	2·7	ND	11·7	tr.	*	0·4	0·6	0·8	0·5	0·4
Potatoes	(R)	0·9	0·1	1·3			0·1	0·1		0·7	<0·05
	(E I)	1·9	ND	3·3	tr.	*	0·3	0·2	tr.	0·5	0·9
	(E II)	0·58	0·03	1·77	tr.		0·08	0·09	0·06	0·35	
Apples	(R)	2·6	0·5	3·7		0·1	0·9	0·5	<0·05	1·0	0·1
	(E I)	2·1	ND	7·3			0·8	0·6	tr.	0·5	0·2
	(T)	6·0	2·3	5·1	0·2	0·2	3·0	1·2	0·3	1·1	+

* No information, whether not determined or below detection limit.
Unless otherwise indicated, absence of a figure means that none was detected.
(E I) = Englyst (1981); (E II) = Englyst *et al.* (1982); (T) = Theander & Åman (1979); (R) = Reistad (present study).
tr. = trace; [x] Cellulose not included; ND = Not determined; [+] All glucose attributed to cellulose.
See also footnotes to Table 1.

TABLE 5
Constituents of Total Non-Starch Polysaccharides (NSP), Calculated as Polymers (Weight %)

Sample		*Total NSP*	*Ur. a.*	*Cellul.*	*Rha.*	*Fuc.*	*Ara.*	*Xyl.*	*Man.*	*Gal.*	*Glu*
Cabbage	(R)	16·5	5·3	6·3	0·2	0·1	2·0	0·6	0·1	1·7	0·2
	(E I)	26·4	9·6	9·3	0·4	*	2·9	1·1	tr.	2·3	0·8
	(E II)	27·6	8·08	9·86	0·90	0·17	3·98	1·52	0·69	2·02	0·38
	(T)	19·8	4·6	9·0	0·6	0·2	1·6	1·5	0·6	1·7	
Cauliflower	(R)	20·7	6·2	7·1	0·3	0·2	3·1	0·9	0·2	2·5	0·2
	(E I)	27·0	5·0	12·5	0·5	*	3·7	1·2	0·3	3·0	0·8
Rutabagas	(R)	18·8	6·9	6·6	0·3	0·1	2·1	0·5	0·1	2·0	0·2
Carrots	(R)	17·5	6·0	5·3	0·3		2·0	0·2	0·1	3·4	0·2
	(E I)	23·4	6·0	9·0	0·4	*	2·1	0·4	tr.	3·9	1·6
	(T)	23·5	7·5	9·1	0·7	tr.	2·0	1·0	0·8	2·3	0·1
Onions	(R)	10·4	3·4	3·5	0·1	<0·05	0·2	0·3	<0·05	2·8	0·1
	(E I)	13·1	3·6	4·0	0·2	*	0·4	0·3	0·1	3·9	0·6
Tomatoes	(R)	11·4	3·9	5·2	0·1		0·6	0·4	0·3	0·8	0·1
	(E I)	24·4	7·4	11·7	0·3	*	0·9	0·7	1·0	1·7	0·7
Potatoes	(R)	3·2	0·6	1·3			0·2	0·1		1·0	<0·05
	(E I)	8·8	1·5	3·3	0·1	*	0·7	0·2	0·1	1·2	1·7
	(E II)	5·14	1·05	1·77	0·13		0·34	0·10	0·06	1·60	0·09
Apples	(R)	10·9	3·3	3·7	<0·05	0·1	1·7	0·5	<0·05	1·5	0·1
	(E I)	14·7	4·1	7·3		*	1·4	0·7	tr.	0·8	0·4
	(T)	16·2	4·6	5·1	0·2	0·2	3·3	1·2	0·3	1·2	0·1

* No information, whether not determined or below detection limit.
Unless otherwise indicated, absence of a figure means that none was detected.
(E I) = Englyst (1981); (E II) = Englyst *et al.* (1982); (T) = Theander & Åman (1979); (R) = Reistad (present study).
tr. = trace.
See also footnotes to Table 1.

TABLE 6

Water-Soluble, Water-Insoluble and Total Non-Starch Polysaccharides (NSP), Calculated as Polymers (Weight %)

Sample		*Dry matter*	*Water-soluble NSP*		*Water-insoluble NSP*		*Total NSP*	
			On fresh basis	*On dry basis*	*On fresh basis*	*On dry basis*	*On fresh basis*	*On dry basis*
Cabbage	(R)	8·2	0·4	5·0	0·9	11·5	1·3	16·5
	(E I)	ND	ND	13·0	ND	13·4	ND	26·4
	(E II)	ND	ND	13·53	ND	14·07	ND	27·60
	(T)	5·5	0·2	2·8	0·9	17·0	1·1	19·8
Cauliflower	(R)	7·8	0·6	7·7	1·0	13·0	1·6	20·7
	(E I)	ND	ND	9·9	ND	17·1	ND	27·0
Rutabagas	(R)	10·3	0·9	9·0	1·0	9·8	1·9	18·8
Carrots	(R)	12·7	1·1	8·5	1·1	9·0	2·2	17·5
	(E I)	ND	ND	11·1	ND	12·3	ND	23·4
	(T)	10·5	0·3	2·9	2·2	20·6	2·5	23·5
Onions	(R)	12·9	0·6	4·9	0·7	5·5	1·4	10·4
	(E I)	ND	ND	7·6	ND	5·5	ND	13·1
Tomatoes	(R)	6·6	0·3	4·3	0·5	7·1	0·8	11·4
	(E I)	ND	ND	10·0	ND	14·4	ND	24·4
Potatoes	(R)	24·0	0·2	0·9	0·6	2·3	0·8	3·2
	(E I)	ND	ND	3·6	ND	5·2	ND	8·8
	(E II)	ND	ND	2·76	ND	2·38	ND	5·14
Apples	(R)	14·9	0·6	4·1	1·0	6·8	1·6	10·9
	(E I)	ND	ND	5·3	ND	9·4	ND	14·7
	(T)	13·6	0·4	2·8	1·8	13·4	2·2	16·2

(E I) = Englyst (1981); (E II) = Englyst *et al.* (1982); (T) = Theander & Åman (1979); (R) = Reistad (present study).
ND = not determined.

The Englyst (1981) method, although quite time-consuming, has the advantage of being conducted in the same tube throughout the major part of the procedure, requiring few quantitative transfers. Determination by difference, as used later in the method of Englyst *et al.* (1982), is not considered an advantage if a complete analysis is the ultimate goal.

Plant polysaccharides are complex structures easily modified by mechanical or chemical treatment, making them behave unlike the native polymer upon subsequent treatment such as precipitation, hydrolysis, etc. Therefore, many considerations have to be taken into account when developing DF analysis procedures, as well as in the treatment of samples prior to analysis.

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