Cell wall polysaccharides determination : comparison of detergent method and direct monomeric analysis

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CELL WALL POLYSACCHARIDES DETERMINATION: COMPARISON OF DETERGENT METHOD AND DIRECT MONOMERIC ANALYSIS

DOSAGE DES POLYOSIDES PARIÉTAUX ; COMPARAISON DE LA MÉTHODE AUX DÉTERGENTS ET DE L'ANALYSE DIRECTE DES MONOMÈRES

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SUMMARY

Cell wall polysaccharides of eight fiber rich materials have been analyzed either by VAN SOEST detergent method or by direct determination of their constitutive monomers. Correspondence between the two methods is not satisfactory. Hemicelluloses and cellulose contents are overestimated by detergent method because of an uncorrect fractionation of cell-wall polysaccharides and, likely, of contamination of residues by non carbohydrate components. The fate of each cell-wall carbohydrate monomer during detergent fractionation points out the limits of this method, even for roughages.

Key-words: *cell-wall carbohydrates, cellulose, hemicellulose.*

RÉSUMÉ

Les polysaccharides pariétaux de huit substrats riches en fibres ont été analysés par la méthode aux détergents de VAN SOEST d'une part et d'autre part par analyse directe de leurs monomères constitutifs. La correspondance entre les deux méthodes n'est pas satisfaisante. La méthode aux détergents surestime les teneurs en hémicelluloses et cellulose en raison d'un mauvais fractionnement des constituants pariétaux et, vraisemblablement, d'une contamination des résidus par des composés non glucidiques. Le suivi, au cours du fractionnement par la méthode aux détergents, des différents oses pariétaux met en évidence les limites de cette méthode pour leur détermination.

Mots clés : *polysaccharides pariétaux, cellulose, hémicellulose.*

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1 - INTRODUCTION

The cell-wall content and composition is an important problem in animal and human feeding. In animal feeding, cell-wall content and characteristics are largely involved in the determination of the energy of the diet. For many years, equations of prediction including parameters linked to cell-wall content of feeds have been used. To this respect, the detergent method developed by VAN SOEST team (GOERING and VAN SOEST, 1970) allowed a more precise prediction than did WEENDE procedure.

New analytical methods have been developed to improve quantification of dietary fibers by simulating enzymic digestion of non cell-wall components of food (PROSKY *et al.*, 1985) and the chemical characterization of their carbohydrate fraction (SAWARDEKER *et al.*, 1965; BLAKENEY *et al.*, 1983). Though more precise, these methods are not adequate for routine analysis and then have not been commonly used. To this respect, the new optimized method developed by HOEBLER *et al.* (1989) allows precise measurement compatible with routine determinations.

The aim of the present work was to compare results of cell-wall polysaccharide determinations obtained on various fiber rich materials by either detergent method of GOERING and VAN SOEST (1970) or the monomeric analysis of HOEBLER *et al.* (1989) and to identify the origin of observed discrepancies.

2 - MATERIALS AND METHODS

Eight fiber rich plant materials (sugar beet pulp, rapeseed hulls, copra meal, alfalfa hay, grass hay, wheat straw, palm meal and sunflower husks) were used. For VAN SOEST determinations, substrates were ground in a hammer-mill fitted with a 1 mm screen. For gas liquid chromatography (GLC) analysis, substrates were ground in a cooled ball-grinder as described by HOEBLER *et al.* (1989).

The fractionation method of GOERING and VAN SOEST (1970) was used to prepare and quantify the different cell-wall fractions of each substrate. Neutral detergent fractions (NDF), acid detergent fractions (ADF) and lignin fractions (lignin) were obtained in duplicate under the action of neutral detergent solution (NDS), NDS then acid detergent solution (ADS) and NDS then ADS then 72% (w/w) sulfuric acid respectively, without any drying between each step.

The monomeric composition of cell-wall polysaccharides was determined by GLC for neutral sugars according to the procedure of HOEBLER *et al.* (1989) and by the colorimetric method of BLUMENKRANTZ and ASBOE-HANSEN (1973), with reference to a galacturonic acid solution, for uronic acids. The cell-wall polysaccharides analysis was performed on each substrate and the corresponding NDF, ADF and lignin fractions.

With the VAN SOEST procedure, hemicelluloses and cellulose content were calculated as NDF-ADF and ADF-lignin respectively. With the procedure of HOEBLER *et al.* (1989), cell-wall glucose was considered as cellulose and other neutral sugars as hemicelluloses. The cell-wall polysaccharides content of hemicelluloses and cellulose fractions of VAN SOEST analysis were calculated as the loss of GLC analyzed carbohydrates during the extraction of ADF from NDF and lignin from ADF respectively.

3 - RESULTS

The cell-wall compositions of raw materials are shown in table 1. Cell-wall content, determined according to GOERING and VAN SOEST (1970) procedure, were in the range 48% and 86%. Except for sugar beet pulp, lignin content was high, especially for rapeseed hulls, sunflower husks and wheat straw. Monomeric analysis of cell-wall polysaccharides showed large differences between each material. Uronic acids, main constituents of pectins, were largely present in sugar beet pulp, alfalfa hay, rapeseed hulls and sunflower husks. Glucose was the most important sugar, except in copra and palm meal which contained large amounts of mannose. Main other sugars were arabinose, especially in sugar beet pulp, xylose, especially in grass hay and wheat straw. Galactose and rhamnose contents were generally low.

The relation between cellulose content determined either by VAN SOEST procedure (X) or by the method of HOEBLER *et al.* (1989) (Y) could be expressed by the following regression: $Y = 0.52\ X + 3.68$ ($r = 0.47$). Figures obtained by the VAN SOEST procedure were always higher than those obtained by HOEBLER *et al.* (1989) method.

The relation between hemicelluloses content determined either by the method of GOERING and VAN SOEST (1970) (Z) or as the total of non-cellulosic neutral carbohydrates (V) was: $V = 1.29\ Z - 10.76$ ($r = 0.75$). For most of studied materials, hemicelluloses contents were higher when calculated by the VAN SOEST method.

Monomeric composition of hemicelluloses and cellulose fractions obtained according to GOERING and VAN SOEST (1970) is shown in table 2. Hemicelluloses mainly contained non cellulosic sugars. However, they could contain large amounts of uronic acids (sugar beet pulp, rapeseed hulls and sunflower husks), glucose (sugar beet pulp, rapeseed hulls, alfalfa hay, grass hay and sunflower husks). The cellulose fractions obtained according to GOERING and VAN SOEST (1970) generally contained large amounts of non cellulosic sugars, either uronic acids (rapeseed hull) or non cellulosic neutral sugars, especially mannose with copra and palm meal. As cellulose is known to only contain glucose, a corrected cellulose content (W) was calculated by correcting VAN SOEST cellulose content for non cellulosic carbohydrates. The relation between this estimation and GLC determined cellulose content was: $X = 0.99\ W - 0.87$ ($r = 0.99$).

Table 1
Cell-wall characteristics of experimental materials (% DM)

		Sugar beet pulp	Rapeseed hulls	Copra meal	Alfalfa hay	Grass hay	Wheat straw	Palm meal	Sunflower husks
VAN SOEST analysis	NDF	48.2	53.0	59.8	44.4	72.3	85.4	86.0	75.6
	ADF	21.6	35.6	31.7	28.9	38.9	54.1	49.9	53.3
	Lignin	2.2	19.0	7.9	6.5	5.0	16.0	12.2	20.2
	Hemicelluloses	26.5	17.3	28.1	15.5	33.4	31.3	36.1	22.4
	Cellulose	19.4	16.7	23.8	22.4	33.9	38.1	37.7	33.1
Monomeric analysis	Uronic acids *	22.0	8.8	0.7	8.4	2.3	2.1	1.5	9.4
	Rhamnose	1.1	0.6	0.1	0.7	0.1	0.1	0.2	0.8
	Arabinose	16.5	5.4	1.8	2.6	2.6	3.1	1.7	2.1
	Xylose	1.5	1.5	1.2	6.8	15.2	16.8	3.6	12.1
	Mannose	1.1	0.5	25.6	1.1	0.5	0.5	41.8	1.0
	Galactose	4.3	1.9	2.8	1.5	1.0	0.7	2.9	0.9
	NC ** neutral sugars	24.4	9.9	31.5	12.7	19.4	21.1	50.1	16.8
	NC ** sugars	46.4	18.7	32.2	21.0	21.6	23.3	51.6	26.2
	Glucose	18.8	8.3	8.2	20.2	29.8	31.4	8.3	22.8

* *Each monomer is expressed in its anhydro form.*
** *Non cellulosic.*

Table 2

Monomeric composition of the carbohydrate fraction of VAN SOEST hemicelluloses and cellulose determination

		Sugar beet pulp	Rapeseed hulls	Copra meal	Alfalfa hay	Grass hay	Wheat straw	Palm meal	Sunflower husks	Mean	Standard error
Hemicelluloses	Uronic acids	17.5	24.2	4.7	15.8	3.9	6.3	4.3	16.1	11.6	2.7
	Rhamnose	1.9	1.8	0.3	0.8	0.3	0.7	2.5	1.3	1.2	0.3
	Arabinose	30.1	28.9	6.4	6.0	7.3	14.5	6.3	7.5	13.4	3.6
	Xylose	3.4	6.7	3.4	26.6	39.9	79.9	8.3	36.3	25.6	9.4
	Mannose	1.8	1.3	71.5	2.5	0.5	2.4	64.1	2.3	18.3	10.8
	Galactose	7.8	11.2	10.5	4.1	2.4	3.8	11.9	2.7	6.8	1.4
	NC * sugars	62.5	74.1	96.9	55.8	54.3	107.4	97.4	66.2	76.8	7.4
	Glucose	37.5	25.9	3.1	44.2	45.7	−7.4	2.6	33.8	23.2	7.4
Cellulose	Uronic acids	8.3	48.6	0.9	14.2	4.4	1.8	0.9	9.7	11.1	5.6
	Rhamnose	0.2	0.4	0.0	0.4	0.4	0.0	0.0	0.3	0.2	0.1
	Arabinose	0.0	0.0	0.2	0.0	0.2	1.9	0.2	0.3	0.3	0.2
	Xylose	2.6	2.3	2.4	9.5	11.2	13.1	4.8	16.0	7.8	1.9
	Mannose	3.5	1.7	66.3	3.0	0.5	0.9	72.2	1.7	18.7	11.1
	Galactose	0.0	0.4	0.4	0.2	0.0	0.1	0.0	0.0	0.2	0.1
	NC * sugars	14.7	53.4	70.4	27.4	16.6	17.9	78.1	28.1	38.3	9.0
	Glucose	85.3	46.6	29.6	72.6	83.4	82.1	21.9	71.9	61.7	9.0

* *Non cellulosic.*

(Each carbohydrate is expressed in percentage of the sum of carbohydrates).

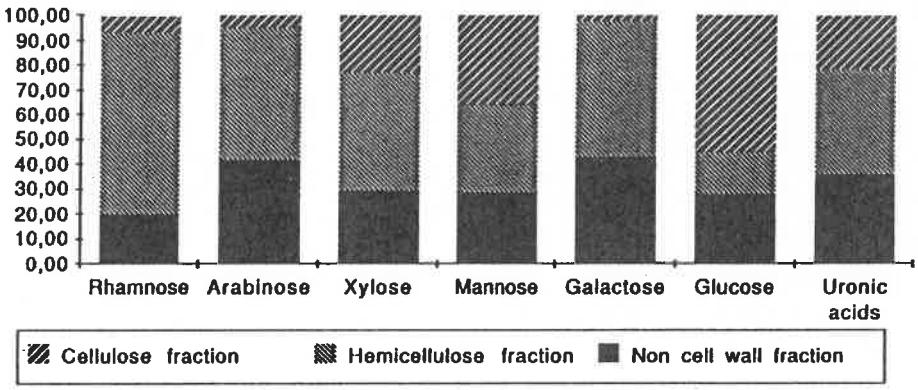

Figure 1
Fractionation of the different cell wall sugars during the Van Soest procedure

Fractionation of neutral and acid cell-wall carbohydrates during the VAN SOEST procedure is represented on figure 1. All the sugars, mainly uronic acids, arabinose and galactose were partly lossed during NDF extraction and were not taken into account as cell-wall sugars. Independently of these losses, rhamnose, arabinose and galactose were mainly found in the hemicellulose fractions of VAN SOEST method. In contrast, an important part of uronic acids, xylose and moreover mannose was found in cellulose fraction.

4 - DISCUSSION

In our experiment, cell-wall glucose was considered as cellulose. Some cell-wall glucose can be of non-cellulosic origin (BUCHALA and FRANZ, 1974): in our case, grass hay and wheat straw contained about 7% of cell-wall glucose which was not present as cellulose but as ß 1-3,1-4 glucans (unpublished results). Similarly, hemicelluloses were calculated as the sum of cell-wall neutral sugars except glucose whereas some neutral sugars (rhamnose, arabinose and galactose) partly come from pectic substances of dicotyledons (CARRE and BRILLOUET, 1986). On the other side, hemicelluloses contain a small fraction of uronic acids (glucuronic acid) which were not taken into account in our calculation. The approximation made on the hemicelluloses content depends on the pectic substances content and could mainly concern sugar beet pulp. On the whole, our calculation method from monomeric analysis of cell-wall polysaccharides could only lead to a small overestimation of cellulose and hemicelluloses contents.

Hemicelluloses and cellulose contents were lower when calculated from GLC analysis than from the VAN SOEST procedure. The monomeric analysis of cell-wall polysaccharides involves an hydrolysis step, generally with sulfuric acid

(HOEBLER *et al.*, 1989). Neutral sugars, particularly pentoses, have been reported to be partially destroyed by sulfuric acid (JARRIGE, 1961): this destruction does not generally exceed 10% and has been found to be about 5%, except for galactose, by NEILSON and MARLETT (1983) with hydrolysis conditions analogous to those we used. Moreover, the greater discrepancies were observed with cellulose whose monomeric sugar (glucose) is known to be resistant to acid hydrolysis. Then, observed differences between results obtained by the two used methodology are not due to an underestimation of cell-wall polysaccharides by GLC analysis but involve an overestimation of these components by the VAN SOEST procedure.

This overestimation is, in part, due to a wrong fractionation of sugars during VAN SOEST procedure. This is quite clear with copra and palm meal whose VAN SOEST cellulose fraction is mainly constituted of mannose. The inaccuracy of VAN SOEST methodology to allow a good fractionation of cell-wall polysaccharides have already been pointed out by MORRISON (1980) who showed that up to 15.4% of VAN SOEST cellulose was not constituted of glucose. Our results show that this contamination can be more important with some materials, even with roughages, like alfalfa or grass hay, though VAN SOEST procedure was developed for this kind of substrates. It should also be noticed that this contamination, even for cellulose fraction, is not only due to neutral sugars, but can be of pectic origin.

As it can be calculated from table 1, the sum of VAN SOEST cellulose and hemicelluloses is generally higher than the determination obtained by monomeric analysis, even if uronic acids are taken into account: it is quite clear that overestimation of cellulose and hemicelluloses content by VAN SOEST procedure is partly due to a contamination of these fractions by non-carbohydrate components: the presence of residual protein in VAN SOEST residues has been reported in roughages (MORRISON, 1980; THEANDER and AMAN, 1980) and in human foods (MARLETT and CHESTER, 1985). However, these contaminations remain generally low, less than 4% in the ADF (MORRISON, 1980); moreover, in the present experiment, the greatest discrepancies between the two methods of determination are observed with substrates containing low nitrogen amounts, such as wheat straw. Other reasons for cellulose and hemicelluloses overestimation by VAN SOEST procedure remain to be found.

The fate of each sugar of cell-wall origin during the VAN SOEST fractionation has been quantified in the present experiment. The important losses of arabinose, galactose and uronic acids observed during the NDF step are in good agreement with previous observations about the important losses of soluble fibres (MONGEAU and BRASSARD, 1986) and more particularly of pectins (ROBERTSON and VAN SOEST, 1981) during NDF preparation. It must be noticed that these losses concern all sugars, even those like xylose and mannose which are not involved in soluble material indicating some solubilization of insoluble cell-wall material. On the other hand, uronic acids which are not solubilized during the obtention of the NDF fraction seem to be highly resistant, even to hot sulfuric acid, and can be found in significant amount in the cellulose fraction of VAN SOEST procedure.

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