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Effect of Hydrolysing and Oxidizing Agents on the Composition and Degradation of Wheat Straw Monosaccharides

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Summary. Wheat straw (WS) was treated with 5% sodium hydroxide, ozone and 5% sulfur dioxide at 70 °C for 72 h, and the effect of treatments on monosaccharide composition and in vitro degradability by rumen microorganisms was studied. The major sugars, glucose and xylose, comprising about 90% of the total monosaccharides in the untreated WS were mainly confined to the cell walls. SO_2 exerted the greatest solubilizing effect, followed by ozone and NaOH; the respective values for the solubilized cell wall polysaccharides were: 26, 12 and 4.4%. One third of the total phenolics was oxidized by ozone, whereas, SO_2 exerted mostly a solubilizing effect on this fraction, converting 75% of it into soluble phenolics. In the NaOH treated WS 41% of the total phenolics were soluble, as compared to 22% in the untreated. The in vitro digestibility of monosaccharides in the untreated WS were initially high: 50% and 58% for xylose and glucose, respectively and 63% to 80% for the minor sugars. The SO_2 treatment resulted in an overall increase in digestibility of monosaccharides with values lying in the range of 90%. Sodium hydroxide was more efficient than ozone in enhancing the degradability of xylan and total sugars. The digestibility of cell wall sugars was increased from 52.4% to 84.4%, 63.4% and 72.3% by SO_2, O_3 and NaOH treatments respectively. Based on the present findings, it appears that wheat straw cell wall components are more sensitive to hydrolytic than to oxidative processes aimed at increasing its degradability by rumen microorganisms. SO_2 exerted on WS a multi-effect which was particularly suitable for increasing the digestibility of monosaccharides.

Introduction

The matrix is usually the main target of attack in pretreatment studies of lignocellulosics. Its partial dissolution creates spaces in the cell wall and renders the cellulose fibers to be readily hydrolysed by cellulases. Much interest has been focused on the chemical and structural organization of the matrix of the plant cell wall unit, since those factors are major determinants of pretreatment effectiveness. Both hydrolysable and oxidative pretreatments have been used to dissolve matrix components in order to increase substrate degradability by fungal cellulase and by rumen microbes, (Millet et al. 1976; Binder et al. 1980; Ben-Ghedalia et al. 1980; Ben-Ghedalia and Miron, 1981).

Oxidative treatments are directed to mainly affect the lignin moiety, whereas hydrolysing agents are expected to influence the lignin-carbohydrate linkages and the hemicellulose. It is suggested that the response of a given lignocellulosic either to hydrolysis or to oxidative treatments reflects the relative importance of the potentially hydrolysable *versus* non-hydrolysable bonds, as biodegradation obstacles in the substrate.

In this study we examined the effect of sulfur dioxide and sodium hydroxide (as hydrolysing agents) and ozone (as oxidizing agent) on the composition and degradability of wheat-straw cell wall monosaccharides by rumen microorganisms.

Materials and Methods

Plant Material and Treatments. Ground (1 mm) wheat straw was used in this study. For treatment with sodium hydroxide, 100 g of milled straw was mixed with 100 ml of 5% NaOH, and kept at room temperature in plastic containers for 1 week, after which the material was freeze-dried and used for analyses. For ozonation, ground wheat straw was moistened to 40% and ozonized in glass columns (80 x 5 cm) until complete decolorization as described by Ben-Ghedalia and Miron (1981). After ozonation the material was freeze dried and used for analyses. For treatment with SO_2, ground wheat straw was moistened to 40% and placed in hermetically sealed glass columns (80 x 5 cm). Gaseous SO_2 was added

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Table 1. The composition (g/100 g OM) of monosaccharides and their distribution (%) in cell walls and cell solubles of wheat straw after treatment with sulfur dioxide, ozone and sodium hydroxide

Monosaccharides	Untreated			Sulfur dioxide			Ozone			5% Sodium hydroxide		
	Composition (g/100 g OM)	Distribution (%)		Composition (g/100 g OM)	Distribution (%)		Composition (g/100 g OM)	Distribution (%)		Composition (g/100 g OM)	Distribution (%)	
		In cell walls	In cell solubles		In cell walls	In cell solubles		In cell walls	In cell solubles		In cell walls	In cell solubles
Glucose	41.6	91.8	8.2	41.4	87.7	12.3	40.6	90.8	9.2	40.9	94.0	6.0
Xylose	20.2	93.8	6.2	20.8	45.9	54.1	18.4	70.7	29.3	21.2	81.7	18.3
Arabinose	2.84	72.7	27.3	3.10	6.6	93.4	3.01	23.0	77.0	3.44	58.6	41.4
Galactose	0.79	65.3	34.7	0.79	7.7	92.3	0.67	36.0	64.0	0.65	44.8	55.2
Mannose	0.40	65.5	34.5	0.42	33.0	67.0	0.36	58.0	42.0	0.32	59.9	40.1
Rhamnose	0.18	54.6	45.4	0.18	0	100	0.22	14.8	85.2	0.18	47.9	52.1
Uronic acids	4.08	82.1	17.9	4.19	41.3	58.7	4.59	56.1	43.9	4.16	69.7	30.3
Total sugars	70.1	90.5	9.5	70.9	67.7	32.3	67.9	79.1	20.9	70.9	86.5	13.5

Table 2. The content of cell walls (CW)[a], CW-glucose polymers[b], CW-non glucose polymers[c], soluble carbohydrates[d], and lignin in wheat straw after treatment with sulfur dioxide, ozone and sodium hydroxide (g 100 g^{-1} OM)

Constituent		Untreated	Treatment		
			SO_2	Ozone	5% NaOH
Cell walls		81.9	55.4	65.2	76.7
CW-glucose polymers		38.2	36.3	36.9	38.4
CW-non glucose polymers		25.2	11.7	16.8	22.8
Soluble carbohydrates		6.7	22.9	14.2	9.5
Lignin (permanganate)		8.31	7.14	6.44	11.2
Total phenolics		17.3	16.4	11.2	16.5
Distribution:	In cell walls	78.2	25.9	68.9	58.8
	In cell solubles	21.8	74.1	31.1	41.2

[a] CW = NDF, neutral detergent fibre = depectinated cell walls
[b] Total CW glucose
[c] Total hemicellulose monosaccharides
[d] Total monosaccharides soluble in neutral detergent

to the straw at a level of 5 g 100 g^{-1} dry matter and then the sealed columns were placed in an oven at 70 °C for 72 h, after which the material was aerated, freeze-dried and used for analyses. Each treatment consisted of four replicates.

Analytical Procedures. Analyses were performed on the four individual samples for each treatment, and are referred to as treatment replicates.

Neutral detergent fiber (NDF-fraction), referred in this study as cell-walls, was prepared according to Goering and Van Soest (1970) and served for sugar analyses. The in vitro digestion procedure of Tilley and Terry (1963) was used to measure the digestibility of monosaccharides. The pattern of digestion was followed by running the incubation with rumen liquor for 6, 12, 24, and 48 h and subsequently with acid pepsin for an additional 48 h. Monosaccharides were determined in whole material, NDF and in the in vitro residues, after acid hydrolysis, as their alditol acetates. The conditions for hydrolysis and preparation of derivatives were those of Sloneker (1972), and for separation by GLC-those of Bacon and Gordon (1980). Uronic acids in hydrolysates were determined colorimetrically as described by Blumenkrantz and Asboe-Hansen (1973). Total phenolics were estimated according to Morrison (1972).

Results

The composition of monosaccharides in wheat straw and their distribution in cell walls and in cell solubles is shown in Table 1. The major sugars, glucose and xylose, comprising about 90% of the total monosaccharides in the untreated WS, were mainly confined to the cell walls. Only 18% of the uronic acids in that material were found in cell solubles, pointing to the low content of pectic substances in WS. The three treatments resulted in a partial solubilization of cell wall sugars with particular effect on the matrix

Table 3. The composition of the cell walls (NDF fraction) after treatment of wheat straw with sulfur dioxide, ozone and sodium hydroxide (g 100 g^{-1} sugars)

Monosaccharides	Untreated	Treatment		
		SO_2	Ozone	5% NaOH
Glucose	60.2	75.7	68.8	62.8
Xylose	29.9	19.9	24.2	28.3
Arabinose	3.26	0.41	1.30	3.31
Galactose	0.81	0.12	0.45	0.48
Mannose	0.41	0.28	0.39	0.31
Rhamnose	0.16	0.	0.06	0.06
Uronic acids	5.28	3.59	4.80	4.74
Total	100	100	100	100

components. SO_2 exerted the greatest effect, followed by ozone and NaOH. The phenomena are summarized and shown in Table 2. The solubilized cell wall material was partly recovered in the soluble sugar fractions. It is assumed that phenolic substances and labile lignins account for the remainder. The content of permanganate-lignin suggested to represent the core and functional lignin was reduced, mainly by the ozone but to a lesser extent also by the SO_2 treatment.

Table 3 presents the monosaccharide profiles of the cell walls in treated and untreated WS. The solubilization of xylans has caused a reduction in cell wall xylose and increased the proportion of glucose particularly in the SO_2 and O_3 treated WS.

The in vitro digestiblity of monosaccharides in whole WS is shown in Table 4. The digestibilities of both major and minor sugars in the untreated WS, were initially high: 50 and 58% for xylose and glucose, respectively and 63% to 80% for the minor sugars. The SO_2 treatment resulted in an overall increase in digestibility of monosaccharides with values lying in the range of 90%. It is assumed that these results are close to the maximal response which is potentially attainable in chemically-treated natural lignocellulosics. Sodium hydroxide was more efficient than ozone in enhancing the in vitro digestibility of glucose, xylose and total sugars. The fact that this difference was not expressed in the organic matter digestibility of the NaOH and O_3 treated WS, implies that soluble products of lignin degradation contributed notably to the digestible fraction of the ozonated WS.

Table 5 presents the degradability of cell wall monosaccharides. Generally, the values were lower for the cell-wall than for the whole plant material. The effect of SO_2 was not confined to the solubilization of cell wall sugars (Ta-

Table 4. In vitro digestibility[a] (%) of monosaccharides of whole wheat straw after treatment with sulfur dioxide, ozone and sodium hydroxide

Monosaccharides	Untreated	SO_2	Ozone	5% NaOH
Glucose	58.1 ± 0.80	88.2 ± 0.96	70.4 ± 0.55	75.1 ± 0.45
Xylose	49.8 ± 1.63	90.8 ± 0.68	58.3 ± 0.86	74.0 ± 0.70
Arabinose	71.9 ± 1.01	96.4 ± 0.38	85.8 ± 0.31	83.2 ± 0.24
Galactose	77.4 ± 0.48	90.0 ± 1.62	74.0 ± 1.65	77.6 ± 0.92
Mannose	79.7 ± 2.40	87.2 ± 1.58	73.1 ± 3.28	64.4 ± 2.33
Rhamnose	64.5 ± 7.0	84.4 ± 3.17	73.4 ± 4.25	52.7 ± 3.36
Uronic acids	63.4 ± 1.32	90.5 ± 0.12	78.5 ± 1.06	69.8 ± 1.02
Total sugars	56.9 ± 1.03	89.5 ± 0.78	68.5 ± 0.21	74.6 ± 0.49
IVOMD[b]	46.2 ± 0.83	81.7 ± 0.36	68.4 ± 0.70	67.5 ± 0.58

[a] Means with their SE
[b] In vitro organic matter digestibility

Table 5. In vitro digestibility[a] (%) of cell wall (NDF) monosaccharides of wheat straw after treatment with sulfur dioxide, ozone and sodium hydroxide

Monosaccharides	Untreated	SO_2	Ozone	5% NaOH
Glucose	55.8 ± 2.08	86.5 ± 1.09	70.1 ± 0.33	74.1 ± 0.47
Xylose	46.5 ± 1.72	80.1 ± 1.49	45.6 ± 1.50	70.5 ± 0.87
Arabinose	61.4 ± 1.39	86.3 ± 2.77	56.0 ± 1.62	78.3 ± 0.41
Uronic acids	55.4 ± 1.57	77.2 ± 0.36	61.0 ± 1.97	59.7 ± 2.92
Total sugars[b]	52.4 ± 1.09	84.4 ± 1.15	63.4 ± 0.24	72.3 ± 0.57

[a] Means with their SE
[b] Including the minor sugars

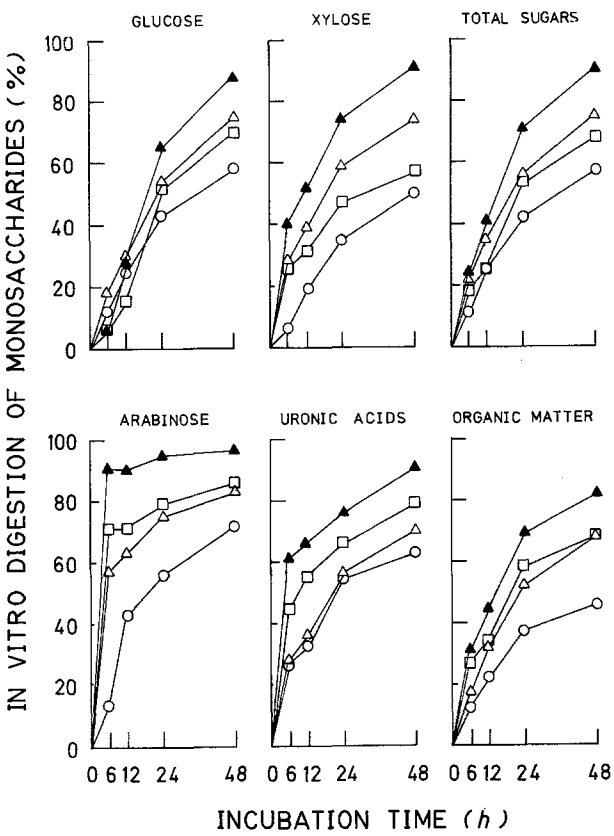

Fig. 1. The pattern of in vitro digestion of monosaccharides in wheat straw treated with: ▲, sulfur dioxide; □, ozone; and △, sodium hydroxide; ○, represents untreated material

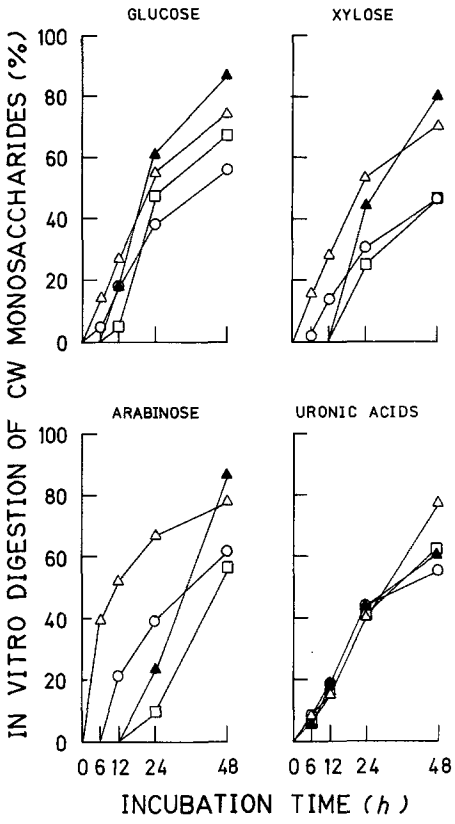

Fig. 2. The pattern of in vitro digestion of cell wall monosaccharides of wheat straw treated with: ▲, sulfur dioxide; □, ozone; and △, sodium hydroxide; ○, represents untreated material

ble 1) but was expressed also in an increase in the degradability of the residual glucose, xylose and total sugars by 55, 72, and 61%, respectively. The digestibility of total cell wall sugars was increased from 52.4% to 84.4, 63.4 and 72.3% by the SO_2, O_3 and NaOH treatments, respectively.

Figures 1 and 2 show the in vitro degradation pattern of monosaccharides and organic matter in whole plant material and in cell walls. Due to the solubilization of cell wall components by SO_2 and ozone, the behaviour of the digestion curves in the cell walls and whole plant material is different. In the cell walls of the SO_2 and O_3 treated WS, there was a lag time of between 6 to 12 h before the degradation of glucose, xylose and arabinose had started. However, since those cell wall sugars in the SO_2 treated material were potentially highly digestible, they reached the highest values of digestibility at 48 h of incubation.

Discussion

Hydrolysis and/or oxidation are the main routes by which biological systems decompose natural lignocellulosics. Most of the readily hydrolysable bonds are found within or associated to the matrix polysaccharides. Including: the labile glycosidic bonds between xylan and arabinofuranosyl residues (Bailey 1973), acetyl-ester bonds (Bacon and Gordon 1980), phenolic esters (Higuchi et al. 1967; Hartley 1973), and lignin-hemicellulose complexes (Morrison 1974; Vered et al. 1981). Notwithstanding, it is widely accepted that most of the chemical bonds linking together the phenolic units in the lignin macromolecule are non-hydrolysable or fairly resistent to mild hydrolysis (Sarkanen 1981). Lignin, on the other hand, is sensitive to oxidation and is being degraded in nature by oxidative processes (Crawford and Crawford 1980; Eggeling and Sahm 1981). It is suggested therefore, that the quantitative ratio of hemicellulose to core lignin in the matrix of a given lignocellulosic, determines whether the substrate is more sensitive to hydrolysis or oxidative treatments. Core lignin is defined in this study, as the non hydrolysable cell wall lignin determined according to the permanganate method (Goering and Van Soest 1970). In Gramineae straws, the ratio of matrix polysaccharides to core lignin, is in the range of 3 to 4 (see Table 2 and Theander and Aman 1980) whereas, in dicotiledonous sources of lignocellulosics (as for example in cotton stalks or grape branches) the ratio is about 1 (Ben-Ghedalia, unpublished). This means that in terms of quantity and space, three quarters of the matrix in the Gramineae straws is composed of and occupied by polysaccharides, mainly branched xylans, representing in terms of sensitivity to hydrolysis, all the above mentioned. This is probably the reason why mild hydrolysing pretreatments are effective when applied

to Gramineae straws (this study and numerous others), while proving completely inefficient when applied to dicotiledonous lignocellulosics (Miron and Ben-Ghedalia 1981; Sneddon et al. 1981).

In this study, SO_2 was the most effective treatment in terms of cell wall dissolution (Table 2) which consequently increased the in vitro digestibility of WS monosaccharides to about 90% (Table 4). It is suggested that the main effect of SO_2 was mediated via the hydrolysis and dissolution of matrix polysaccharides (Table 1); this effect being complemented by solubilization of cell wall phenolics (Table 2). SO_2 is able to hydrolyse labile ether bonds within the lignin molecule (Sarkanen 1981) and to sulfonate the "new born" hydroxyls as well as others attached to the aliphatic "tail" of the phenolic units (Forss et al. 1966). And since the lignin molecules of WS are much smaller than those found in woody materials (Ben-Ghedalia, unpublished), they became easily soluble (as sulfonated lignins) under the mild conditions of this study. Thus, it is assumed that the effect of SO_2 on the lignin of WS was a combined action of hydrolysis and sulfonation.

The primary effect of O_3 was expressed in partial oxidation of the phenolic fraction (Table 2) and the consequent release of the lignin associated carbohydrates. Secondary hydrolysis of matrix polysaccharides by the organic acids produced during ozonation (Ben-Ghedalia et al. 1982) cannot be ruled out. However, the comparison of in vitro digestibilities of sugars in the O_3 and NaOH treated straw (Tables 4 and 5, Figs. 1 and 2) points to the somewhat better effect of the NaOH treatment, particularly with respect to xylan degradability. This might lead to the conclusion that hydrolytic and swelling effects, as probably exerted by NaOH (Chesson 1981; Bacon et al. 1981) are of major importance when referred to pretreating WS, exceeding even the partial dissolution of cell wall material. SO_2 at 70 °C exerts a multi-effect which is particularly suitable for tackling the chemical and structural problemacy of the WS cell wall, in order to enhance its degradability by biological systems.

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