Silage Fermentation and *in Vitro* Degradation of Monosaccharide Constituents of Wheat Harvested at Two Stages of Maturity

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Wheat plants (*Triticum aestivum*) were harvested at the bloom and soft-dough stages and ensiled in laboratory silos, and the effect of stage of maturity on the recovery of soluble and cell wall components and on the *in vitro* digestibility of monosaccharide residues was examined. A total of 8–9% neutral detergent fiber (NDF) monosaccharides residues was solubilized and partly utilized during ensilage. Glucose and galactose were the most fermentable sugars among the ND soluble monosaccharides. Fructose was equally fermented in both silages, but the higher recovery of reducing sugars in the soft-dough silage as compared with the bloom silage (44.1 *vs* 26.8) indicates the possible participation of starch in the fermentation process of the former. Generally, the silages were less digestible than the source materials, but the difference was larger in the bloom treatment. Thus, a gap of 8.1 percentage units in total NDF monosaccharide digestibility (*D*) between the source materials was reduced to 5.5 units between the silages. The decline in the *D* values of the monosaccharide residues of the whole plant following ensilage was 11.5 and 6.0 percentage units for the bloom and soft-dough stages, respectively. The *D* of total monosaccharide residues of whole plant material was equal within the source materials but was 5.3 percentage units higher in the soft-dough silage than in the bloom one. Digestibility measurement at the monosaccharide level of source materials and silages offers a sharp tool for determining the optimal stage for harvesting wheat for silage.

Keywords: *Wheat silage; stage of maturity; monosaccharide digestibility; monosaccharide fermentation*

INTRODUCTION

The optimal stage for harvesting wheat for silage is a major issue in semiarid areas in which wheat is grown as a winter crop. It has been shown recently (Ben-Ghedalia et al., 1995) that the decrease in neutral detergent fiber (NDF) monosaccharide digestibility between the bloom and soft-dough stages is compensated by an increase of 36% in the yield of digestible dry matter. Nevertheless, the concern of getting a forage that is a combination of "straw and starch" is raised occasionally as the argument in favor of harvesting wheat forage at the bloom stage of maturity (Arieli and Adin, 1994). Starch accumulated in the spike provides an abundant source of fermentable carbohydrates for successfully ensiling the soft-dough plants. However, the entire "arsenal" of silage fermentable carbohydrates found in wheat harvested at the bloom stage has not been fully discovered yet. For instance, the more fragile cell walls (CW) and labile CW components found in the younger wheat plant may participate in the fermentation process, and if they do, is their contribution important or necessary? There are numerous studies on the patterns of silage fermentation of wheat harvested at different stages of maturity (Ashbell et al., 1983; Ashbell and Kashanchi, 1987; Weinberg et al., 1991); none of this information is related, however, to the fate of CW constituents. Moreover, the role of CW monosaccharide components in the process of silage fermentation has been investigated in grasses (Morrison, 1979) and in lucerne (Ben-Ghedalia and Yosef, 1989a,b; Campbell et al., 1990), but relevant information on wheat does not exist yet.

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Chemical and *in vitro* investigation of fresh plant material (Ben-Ghedalia et al., 1995) is important for following the changes occurring in the wheat plant during maturation. Plant material, however, is modified during ensilage. To obtain information required for determining the optimal stage of harvesting, both source materials and silages should be evaluated.

The objectives of this experiment were to study the effect of maturation on the *in vitro* degradability of carbohydrates in wheat silages and in the respective source materials and to assess the recovery of soluble and CW monosaccharide components following the ensilage of wheat harvested at bloom and soft-dough stages of maturity.

MATERIALS AND METHODS

Plant Material and Ensiling Procedure. Winter wheat (*Triticum aestivum* var. Ariel) plants were harvested (10 cm above ground) from a commercial field at bloom and after 19 days at the soft-dough stage. Shortly after harvest, plants were transferred to the laboratory and chopped into pieces 1–2 cm in length, and a portion representing the source material was kept at −20 °C until analyzed. The second portion of the chopped soft-dough material, containing 35% dry matter (DM), was ensiled directly in hermetically sealed 2-L laboratory glass silos, whereas the bloom stage material, containing 25% DM, was sun wilted to reach 35% DM prior to ensiling. Both materials were ensiled, four replicates per treatment, for 60 days at 21–23 °C. After this period, the silos were opened for assessing the recovery of fructose, total reducing sugars, neutral detergent soluble (NDS) monosaccharides, and NDF monosaccharide components and to determine the profile of fermentation products and the *in vitro* digestibility of monosaccharide constituents.

Analytical Procedures and *in Vitro* Determination. DM and organic matter (OM) contents were determined according to AOAC methods (Association of Official Analytical

0021-8561/95/1443-2428$09.00/0

Table 1. Concentration (Grams per 100 g of DM) of Monosaccharide Residues and Their Distribution (Percent) in Neutral Detergent Fiber (NDF) and Neutral Detergent Solubles (NDS) of Wheat Plants Harvested at Two Stages of Maturity

	bloom stage			soft-dough stage		
		distribution in			distribution in	
monosaccharide	composition	NDF	NDS	composition	NDF	NDS
glucose	31.4	83.3	16.7	34.6	64.3	35.7
xylose	14.1	100	0	11.8	100	0
arabinose	2.36	95.3	4.70	2.69	92.1	7.91
uronic acids	2.87	60.1	39.9	2.40	59.7	40.3
galactose	0.94	49.9	50.1	0.79	62.3	37.7
mannose	0.44	38.0	62.0	0.48	41.0	59.0

Table 2. Composition (Grams per 100 g of DM) and pH of Wheat Source Materials (SM) and Silages Harvested at Bloom and Soft-Dough Stages

	bloom		soft-dough	
component	SM	silage	SM	silage
pH		4.47		4.24
lactic acid		3.18		4.28
acetic acid		2.77		3.16
isobutyric acid		0.12		0.24
butyric acid		0.20		0
fructose	11.7	5.64	14.6	6.49
reducing sugars	12.7	3.84	10.3	5.20
NDF[a]	58.4	62.3	50.9	53.5
NDF glucose	26.2	28.5	22.3	24.5
hemicellulose[b]	18.7	18.3	16.4	16.5
lignin	5.86	7.12	5.66	6.56
silica	3.32	3.82	3.68	4.64

[a] Neutral detergent fiber. [b] Based on monosaccharide analysis.

Table 3. Recovery after 60 days of Fermentation (Percent of Pre-ensilage Component)[a] of Neutral Detergent Fiber (NDF) Monosaccharide Components in Wheat Silages Harvested at Two Stages of Maturity

monosaccharide	bloom	soft-dough	SEM
glucose	95.9	95.0	1.41
xylose	87.0	88.7	1.54
arabinose	76.0	80.9	1.90
uronic acids	89.6	90.4	1.18
galactose	70.6[a]	77.2[b]	1.65
mannose	59.4[a]	89.5[b]	1.47
total NDF monosaccharides	91.4	91.7	1.31

[a] At day = 0, each component was regarded as 100%. Values in the same line marked by different superscripts are significantly different, $P < 0.05$.

Chemists, 1984). Silage samples were extracted with distilled water at 0 °C; this extract was used for pH, volatile fatty acids (VFA) (Ben-Ghedalia and Yosef, 1989c), lactic acid (Pryce, 1969), and reducing sugars (Miller, 1959) determination.

Another portion of the silages and the corresponding source materials was freeze-dried, ground to 1 mm, and used for measuring and preparing NDF according to the method of Van Soest et al. (1991), employing the amylase procedure. Permanganate–lignin and silica were determined according to the method of Van Soest et al. (1991).

To extract fructose, 5 g DM of freeze-dried and ground samples of source materials and silages underwent extraction with 100 mL of 0.1 N HCl for 1 h. Total fructose in the extracts was determined according to the procedure of Boratynski (1984).

Monosaccharide components of the freeze-dried source materials and silages and their NDF preparations were determined after hydrolysis with 24 N H_2SO_4 for 1 h at 21 °C followed by 1 N H_2SO_4 for 5 h at 100 °C, as described by Ben-Ghedalia and Miron (1984). The free sugars released were converted to alditol acetates and determined by GLC (Blakeney et al., 1983). Uronic acids in the hydrolysates were determined colorimetrically (Blumenkrantz and Asboe-Hansen, 1973).

In vitro digestibility of source materials and silages was determined (in four tubes = replicates) according to the two-stage fermentation technique of Tilley and Terry (1963). Residual DM, NDF, and monosaccharide components remaining in each fermentation tube were determined as described above.

Results were analyzed statistically by using a completely randomized design (Little and Hills, 1978) consisting of two stages of maturity (treatments), with four replicates (silos) per treatment.

RESULTS AND DISCUSSION

Silage Fermentation. The monosaccharide composition of the source materials is shown in Table 1. The major change between the bloom and soft-dough stages was expressed in a doubling of the proportion of NDS glucose due to starch accumulation in the spikes. Table 2 presents the composition of silages and source materials. Theoretically, starch may participate in the process of silage fermentation (Henderson, 1993). However, the high concentrations of fructose (11.7 and 14.6%) and reducing sugars (10.6 and 12.7%) in the wheat plants prove that, in this study, there was no shortage in readily fermentable carbohydrates. Both silages were good; pH values of 4.47 and 4.24 for the bloom and soft-dough silages reflect the concentrations of lactic acid, 3.18 and 4.28%, respectively. Along with the decrease in the concentration of the fermentable carbohydrates, there was an increase in the content of the fibrous materials, in both silages. This point is worth mentioning since it is probably associated with changes in digestibility following ensilage.

The participation of NDF monosaccharide constituents in the process of silage fermentation is shown in Table 3. The general view is that NDF monosaccharides contribute very little to that process. This is, however, based on very sparse information. For instance, 100% recovery of NDF glucose and xylose residues after silage fermentation was found with lucerne (Ben-Ghedalia and Yosef, 1989a,b), whereas Morrison (1979) reported losses of 5% for cellulose and 10–20% for hemicellulose in grasses. Table 3 is the first to show the relevant data on wheat. The general picture is that NDF monosaccharide residues are being solubilized and, most likely, partly utilized during the ensilage of wheat. The extent of solubilization was 4–5, 11–13, 19–24, and 10% for NDF glucose, xylose, arabinose, and uronic acids, respectively. A total of 8–9% NDF monosaccharides were solubilized.

The recovery of NDS monosaccharides, fructose, and total reducing sugars is shown in Table 4. Glucose and galactose were the most fermentable sugars among the NDS monosaccharides, having the major and significant contribution in the bloom wheat silages. Since during ensilage there is a transfer of monosaccharides from NDF to NDS (Table 3), it is difficult to assess precisely the contribution of NDS sugars to the fermentation

Table 4. Recovery after 60 days of Fermentation (Percent of Pre-ensilage Component)[a] of Neutral Detergent Soluble Monosaccharides (NDS), Fructose, and Total Reducing Sugars in Wheat Silages Harvested at Two Stages of Maturity

component	bloom	soft-dough	SEM
glucose	16.4[a]	56.2[b]	5.41
xylose	147	320	59.0
arabinose	183	152	15.5
uronic acids	86.3	87.5	2.92
galactose	35.2[a]	76.4[b]	3.99
mannose	98.7[a]	594[b]	27.6
total NDS monosaccharides	36.6[a]	78.6[b]	6.08
fructose	42.7	38.9	2.19
total reducing sugars	26.8[a]	44.1[b]	3.06

[a] At day = 0, each component was regarded as 100%. Values in the same line marked by different superscripts are significantly different, $P < 0.05$.

process. Soluble xylose and arabinose can participate in silage fermentation. Chamberlain (1988) had shown that 50–70% of added xylose was fermented during ryegrass fermentation, and in our previous studies (Ben-Ghedalia and Yosef, 1989a,b) up to 80% of the indigenous ND arabinose found in lucerne was fermented during ensilage. The high recoveries of these ND sugars in the present study (Table 4) indicate that their role in ensilage was very limited, which is not surprising in view of the high concentrations of fructose and NDS glucose found in the source materials (Tables 1 and 2). Lactic acid bacteria are devoid of pectinases (Ben-Ghedalia et al., 1993), and therefore galacturonic acid, the major building block of the rhamnogalacturonans, is not consumed during ensilage (Ben-Ghedalia and Yosef, 1989a; Ben-Ghedalia et al., 1991, 1993). Mannose is usually a minor monosaccharide component found largely in the NDS and may be consumed during silage fermentation, particularly when there is a shortage in the preferable substrates (Ben-Ghedalia and Yosef, 1989a,b). The significantly very high recovery of mannose in the soft-dough silage (600%, due to an increase of its content from 0.48 to 2.20%) was probably a result of microbial biosynthesis. Fructose was equally fermented in both silages; however, the significantly different recoveries of total reducing sugars found in the silages of this study, and the mannose phenomenon mentioned above, hint at the possibility of different fermentation patterns. In this context, the possible participation of starch in the ensilage of the soft-dough plants cannot be excluded.

***In Vitro* Digestibility.** Carbohydrate digestibility was determined recently in fresh wheat plants harvested at the bloom and soft-dough stages (Ben-Ghedalia et al., 1995). Wheat is usually preserved as silage; however, in view of the changes occurring in the carbohydrate fractions during ensilage (Tables 3 and 4), it is important to compare the digestibility (D) of these fractions between source materials and silages and between the silages themselves. This is shown for the NDF monosaccharide residues in Table 5. Generally, silages were less digestible than the source materials, but the difference was larger in the bloom than in the soft-dough treatment. Except for NDF galactose, the decline in the digestibility of all the other NDF monosaccharides following ensilage was greater in the bloom stage than in the soft-dough stage. The decline in total NDF monosaccharide D was 5.1 and 2.5 percentage units in the bloom and soft-dough stages, respectively. Thus, a gap of 8.1 D percentage units in total NDF

Table 5. *In Vitro* Digestibility (Percent)[a] of NDF Monosaccharides of Wheat Source Materials (SM) and Silages Harvested at Two Stages of Maturity

	bloom		soft-dough		
component	SM	silage	SM	silage	SEM
glucose	66.4[a]	62.4[b]	58.2[c]	55.6[d]	0.54
xylose	63.4[a]	56.9[b]	53.0[c]	51.8[c]	0.61
arabinose	78.8[a]	71.6[c]	77.0[b]	72.4[c]	0.35
uronic acids	67.1[a]	62.9[b]	61.3[b]	58.3[c]	0.53
galactose	85.8[a]	78.1[c]	79.8[b]	70.8[d]	0.34
mannose	86.2[a]	77.2[c]	84.7[b]	85.0[b]	0.24
total NDF monosaccharides	66.4[a]	61.3[b]	58.3[c]	55.8[d]	0.55
NDF	63.3[a]	57.8[b]	56.3[b]	50.8[c]	0.62

[a] Values in the same line marked by different superscripts are significantly different, $P < 0.05$.

Table 6. *In Vitro* Digestibility (Percent)[a] of Whole Plant Monosaccharide Residues of Source Materials (SM) and Silages Harvested at Two Stages of Maturity

	bloom		soft-dough		
component	SM	silage	SM	silage	SEM
glucose	73.1[a]	60.4[c]	74.9[a]	65.7[b]	0.60
xylose	65.5[a]	55.3[c]	58.8[b]	57.1[bc]	0.82
arabinose	79.1[a]	71.4[c]	79.6[a]	75.5[b]	0.79
uronic acids	75.2[a]	69.7[b]	73.0[a]	67.6[b]	0.91
galactose	82.2[a]	62.6[d]	75.3[b]	67.2[c]	0.82
mannose	87.7[b]	89.6[b]	90.6[b]	97.2[a]	0.97
total monosaccharides	71.7[a]	60.2[c]	71.6[a]	65.5[b]	0.60
organic matter	69.2[a]	59.0[c]	70.5[a]	65.6[b]	0.51

[a] Values in the same line marked by different superscripts are significantly different, $P < 0.05$.

monosaccharide D between the source materials was reduced to 5.5 D percentage units between the silages. This picture is not reflected in the NDF fraction, which comprises also the phenolic component. Therefore, in this study as in many others, NDF monosaccharide digestibility data are better measurement for differentiating treatment effect.

The D values of the monosaccharide residues in whole plant material is shown in Table 6. The presence of starch in the soft-dough source material compensated for the decline in NDF glucose D due to maturation. The decline in D of total monosaccharide residues of the whole plant following ensilage was 11.5 and 6 percentage units for the bloom and soft-dough stages, respectively. Thus, while the D of total monosaccharide residues was equal within the source materials, it was higher by 5.3 percentage units in the soft-dough silage than in the bloom one. Accordingly, the D of the individual monosaccharide components except the uronic acids was higher in the soft-dough silage than in the bloom silage. It was found in a recent study that between the bloom and soft-dough stages there is a DM yield increase of 36% (Ben-Ghedalia et al., 1995). In the present study, carbohydrate D of the soft-dough silage was 9% higher than that of the bloom silage; thus, by harvesting wheat for silage at that stage, the yield gain in terms of digestible energy would be more than 45%.

For reaching the right conclusion regarding the optimal stage of harvesting wheat for silage, it is recommended that both source materials and silages be evaluated at the monosaccharide level.

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Received for review March 7, 1995. Accepted June 12, 1995.[⊗]

JF950137M

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1995.