Measuring Lignin and Neutral Sugars in Neutral Detergent Soluble and Insoluble Fractions of Human and Rat Feces

Edward S. Kris‡ and Judith A. Marlett*

Department of Nutritional Sciences, University of Wisconsin—Madison, 1415 Linden Drive, Madison, Wisconsin 53706

The aims of this research were to evaluate modifications of the neutral detergent fiber (NDF) procedure as a method of fractionating neutral sugars in feces and to compare several measures of fecal crude lignin. Fecal samples from humans and rats fed wheat bran or cellulose were analyzed. Fecal neutral sugar concentration ranged from 19% for human wheat bran to 65% for rat cellulose, with the composition reflecting the dietary fiber source; 81–98% of the fecal neutral sugars were recovered in the NDF residue. Whether the NDF extraction was conducted at 100 °C for 1 h or at 40 °C for 24 h, addition of $NaSO_3$ to the neutral detergent solution lowered NDF yield and nitrogen content without altering the NDF neutral sugar content. Except for the human cellulose feces analyzed with $NaSO_3$, temperature had no effect on fecal NDF. The lignin contents of the cellulose samples were generally similar when measured as acid detergent fiber crude lignin (AL) or permanganate lignin (PL) or as material remaining after Saeman acid hydrolysis of NDF analyzed at both temperatures with or without $NaSO_3$. PL and AL from wheat bran feces were comparable to or lower than those obtained by acid hydrolysis of NDF residue. Klason lignin remaining after acid hydrolysis of feces was always higher than the other measures of lignin.

Determining the function and mechanisms of action of dietary fiber in the gastrointestinal tract requires the identification and quantification of the components of dietary fiber within the gastrointestinal tract and in the feces as well as of the components of the dietary fiber in the food source. The neutral detergent fiber (NDF) procedure, originally developed by Van Soest and Wine (1967) to measure fiber in forages, has been used frequently to measure dietary fiber in feces because the detergent solubilizes bacteria. In previous work from this laboratory, however, the sum of two major components of NDF, neutral sugars and lignin, was 30–50% less than the NDF weight; this was true even though the crude lignin fraction was much larger than that predicted from the dietary intake (Slavin et al., 1983). The apparent negative fiber digestibilities, particularly of the lignin fraction, reported by several laboratories [see Marlett and Johnson (1985) for review] suggest that some material is being recovered in fecal fiber by the NDF method that is not part of the ingested food fiber.

Lignin is a complex group of aromatic polymers formed by the condensation of the aromatic alcohols: cinnamyl, guaiacyl, and syringyl alcohols (Southgate, 1981). Various methods have been developed to estimate the lignin content of foods: digestion with 72% sulfuric acid (Goering and Van Soest, 1970), permanganate oxidation (Goering and Van Soest, 1970), oxidation/methylation (Theander et al., 1977), and the acetyl bromide method (Johnson et al., 1961). None of the methods measures lignin specifically. Fecal lignin has been most frequently measured as the material remaining after Saeman acid hydrolysis or after treatment of NDF or acid detergent fiber (ADF) with sulfuric acid at room temperature. Lignin measured in ADF is termed Klason lignin by Robertson and Van Soest (1981). Residue remaining after Saeman acid hydrolysis of food fiber is termed Klason lignin by Theander (Theander and Westerlund, 1986).

When lignin is measured in fecal NDF or ADF, a significant amount of nitrogen is recovered in the lignin. The extent of this nitrogen contamination is reduced by the presence of sodium sulfite in the detergent solution (Marlett and Johnson, 1985). One possible source of this nitrogen might be Maillard products, which are condensation products of sugar and protein produced upon heating in the presence of adequate moisture (Robertson and Van Soest, 1981). One of the objectives of our research was to determine the effectiveness of sodium sulfite at either low or high temperature to remove nitrogen from the NDF residue of several types of fecal samples. The second objective was to compare several measures of crude lignin: the acid-insoluble residue after a Saeman hydrolysis of feces, Klason lignin; the acid-insoluble residue after Saeman hydrolysis of NDF, NDF crude lignin; acid detergent fiber (ADF) crude lignin; and permanganate lignin.

Most methods developed to measure fiber in foods will recover from excreta, in addition to dietary fiber, unknown amounts of endogenously secreted mucins, bacteria, and bacterially synthesized exopolysaccharides. The exception is the NDF method, which extracts these products, recovering as NDF the undigested cellulose, hemicellulose, and lignin (Robertson and Van Soest, 1981; Slavin et al., 1981). The NDF procedure also extracts soluble dietary fiber components of foods, e.g., pectins, gums, and a portion of the hemicelluloses. However, because the soluble fiber fraction is highly fermentable, it is generally thought that only negligible amounts survive the gastrointestinal tract. Separating fecal carbohydrates into NDF residue and NDF filtrate would provide an operational division of the carbohydrate into plant and bacterially derived components, respectively. This hypothesis was the basis for the third objective: the measure-

‡ Present address: Eppley Institute for Research in Cancer and Applied Disease, 42nd and Dewey Ave., Omaha, NE 68105.

0021-8561/90/1438-1218$02.50/0

ment of neutral sugars in feces, NDF residue, and NDF filtrate.

EXPERIMENTAL METHODS

Fecal samples were collected from humans and rats fed diets containing wheat bran or cellulose. Seven women consumed nutritionally adequate diets consisting of a mixture of foods and 16 g/day of Solka Floc (James River Corp., Berlin, NH) (Slavin and Marlett, 1980) for 4 weeks; two men and five women consumed mixed ad libitum diets and 30 g/day of soft, white wheat bran (American Association of Cereal Chemists soft white wheat bran, AACC, St. Paul, MN) (Balasubramanian et al., 1987) for 3 weeks. Subjects in the Solka Floc study consumed constant weighed diets of ~400 g (dry weight) daily; the approximate concentration of the Solka Floc supplement, therefore, was 4%. Since subjects in the wheat bran study consumed ad libitum diets, the daily dry food intake is not known. By use of an estimated range of dry food intake for the general population of 400–600 g, the concentration of the wheat bran supplement to the daily diet was 5.0–7.5%. Composites of blended, lyophilized feces from the last 5 days of each study were prepared by combining 2% of the dry output from each subject. Rats (8/group) were fed for 3 weeks the AIN-76A purified rodent diet containing 5% (by weight) cellulose (Celluflour, 160390, Teklad, Madison, WI) or 11% fiber provided by 25% wheat bran (AACC), similar to diets used previously in this laboratory (Balasubramanian, 1987; Lopez-Guisa et al., 1988). The dietary fiber contents of the cellulose and wheat bran were determined by the Theander method A (Theander and Westerlund, 1986) and were 46% for the wheat bran, 90% for the Solka Floc, and 90% for the cellulose fed to the rats. Feces were collected during approximately the last half of each rat study and blended and lyophilized; a composite was prepared by combining 10% of total output from each rat. All analyses, except as noted, were done in triplicate.

Fecal Analysis. Fecal samples were analyzed for neutral sugar content and composition by gas chromatography (HP5890, Hewlett-Packard Co., Avondale, PA) after acid hydrolysis. Samples (50 mg) were acid hydrolyzed by a modified Saeman hydrolysis that consisted of two steps; first, the sample was treated with 1 mL of 12 M H_2SO_4 for 1 h at 25 °C, and then, after dilution to 1 M H_2SO_4 with distilled water, the sample was heated to 121 °C for 1 h. After hydrolysis, 1 mg of allose (Sigma Chemical Co., St. Louis, MO) was added to the cooled samples as an internal standard. Hydrolyzed samples were filtered through tared glass filter paper (Whatman GF/A), and aliquots (0.9 mL) of the filtrates were analyzed for neutral sugars as the alditol acetates (Englyst and Cummings, 1984). The filter paper containing the acid-insoluble residue was dried overnight at 100 °C and weighed to determine Klason lignin (Theander and Westerlund, 1986). Alditol acetates were separated on a 30 m × 0.25 mm i.d. fused silica capillary column coated with SP2380 (Supelco, Inc., Bellefonte, PA). The carrier gas was helium at a flow rate of 0.6 cm^3/min. The injection and detector temperatures were 250 °C; the oven temperature was 240 °C.

Fecal NDF Analysis. Fecal samples (≃1.0 g) were also analyzed for NDF content (Goering and Van Soest, 1970). NDF analyses were performed with 100 mL of NDF solution in four ways: refluxing for 1 h at 100 °C, which is the standard method, or incubation for 24 h at 40 °C; both analyses were conducted with 0.5 g of sodium sulfite and without sodium sulfite in the detergent solution. After incubation of 40 °C, samples were heated to 100 °C in ≤3 min to facilitate filtering. All NDF residues were filtered through tared Gooch crucibles (50 mL, high form, coarse porosity) under vacuum and washed well (~900 mL of 100 °C distilled water). The tared glass wool pad (~400 mg) that was used as a filtering aid (Brauer, et al., 1981) and contained the NDF residue was placed in a tared 100-mL beaker, dried overnight in a vacuum oven (with P_2O_5 at 25 °C), and then placed in a 100 °C oven for 1 h for hot weighing (Brauer et al., 1981). Residual NDF not removed with the glass wool pad but retained on the crucible was determined by drying the crubibles overnight (100 °C) and then weighing them. The glass wool containing the NDF residue was hydrolyzed by the modified Saemen hydrolysis described above. The amount of 12 M acid was 1 mL/50 mg of NDF, which is comparable to the 8 g of acid/g of glass wool used previously in this laboratory (Slavin et al., 1983). Hydrolysates were filtered through tared Gooch crucibles to recover the glass wool and the acid-insoluble residue; these were ashed for 8 h at 550 °C (Goering and Van Soest, 1970) and then placed in the 100 °C over for 1 h and hot weighed. The loss in weight with ashing was the measure of NDF crude lignin recovered from fecal NDF. Duplicate NDF residues were also generated for Kjeldahl nitrogen analysis.

Fecal NDF Filtrate Analysis. A 250-mL aliquot of the combined NDF filtrate and washes was dialyzed against distilled water for 72 h by using 12 000–14 000 molecular weight cutoff tubing (Spectropor membrane tubing, Spectrum Medical Industries, Inc., Los Angeles, CA) and then lyophilized. Twenty-five milligrams of the dried filtrate was hydrolyzed as described above and neutralized with powdered barium carbonate (5.213 g/mL of 12 M sulfuric acid) when samples had cooled to <60 °C; the samples were centrifuged to remove insoluble barium sulfate (Neilson and Marlett, 1983). Barium carbonate was used instead of ammonium hydroxide to allow lyophilization of the sample after neutralization. The hydrolysate was derivatized as described previously, except that the sample was lyophilized after reduction with sodium borohydride to concentrate the sugars. The entire sample was dissolved in 0.2 mL of water, and the remainder of the derivatization was the same as described previously (Englyst and Cummings, 1984). Neutral sugar losses during acid hydrolysis, which were determined by subjecting standard neutral sugars (Sigma) to the same treatment, were used to correct the gas chromatographic measurement of neutral sugars. Recoveries of standard neutral sugars were (%, mean ± SD, n = 4) as follows: rhamnose, 85.8 ± 2.3; fucose, 88.0 ± 2.3; ribose, 64.2 ± 2.1; arabinose, 82.1 ± 1.3; xylose, 75.0 ± 1.4; mannose, 92.7 ± 1.1; galactose, 91.7 ± 0.6; and glucose, 92.9 ± 3.0. Sugar data were expressed as the polysaccharide (x0.9).

Klason Lignin and Permanganate Lignin Analysis. Fecal samples were also analyzed for ADF and the fecal ADF used for ADF crude lignin and permanganate lignin determinations as described by Robertson and Van Soest (1981).

Statistics. Statistical differences were determined with least-squares analysis of variance using the General Linear Model procedure and predetermined single degree of freedom contrasts and predicted differences. Predicted differences from least-squares analysis were used for comparisons within each type of fecal sample: human wheat bran, rat wheat bran, human cellulose, and rat cellulose (Ray, 1982).

RESULTS AND DISCUSSION

The neutral sugar content of the feces varied substantially, from 19% of fecal dry weight for the sample collected from adults consuming 30 g of wheat bran daily to 65% of the feces from rats fed 5% cellulose (Table I). In both species the neutral sugar content of the cellulose-containing feces was higher than that of the feces collected during wheat bran consumption.

The neutral sugar composition of the feces generally reflected the dietary fiber source that was ingested (Table I). Glucose accounted for 91% and 92% of the fecal neutral sugars in the humans and rats consuming cellulose, respectively. In contrast, the sum of arabinose and xylose, which accounts for about two-thirds of the fiber-derived neutral sugars in wheat bran (Neilson and Marlett, 1983), was 50% and 59% of the total neutral sugars in human and rat wheat bran feces, respectively. Rhamnose, fucose, and mannose were detected in all samples, although they were generally below the limits for quantitation, which was ≤0.5% for the analytical procedure we used. One to two percent of the fecal neutral sugars were ribose. Recovery of fecal neutral sugars as the sum of the sugars in the NDF-insoluble and NDF-soluble fractions was good, ranging from 88–89% for the rat cellulose and human wheat bran feces to 100% for the human cellulose and rat wheat bran samples (Table I).

Mean fecal dry weights were used to estimate the amount

Table I. Measurement and Fractionation of Neutral Sugars in Human and Rat Feces[a]

	% dry weight of feces				
	arabinose	xylose	galactose	glucose	total
human					
wheat bran					
feces	4.6 ± 0.2	5.1 ± 0.2	1.0 ± 0.1	8.6 ± 0.2	19.3 ± 0.6
NDF insoluble	4.2 ± 0.2	4.9 ± 0.1	tr[b]	6.5 ± 0.3	15.6 ± 0.6
NDF soluble	tr[b]	tr[b]	0.6 ± 0.1	1.0 ± 0.0	1.6 ± 0.1
cellulose					
feces	0.8 ± 0.3	2.8 ± 0.2	0.8 ± 0.0	43.0 ± 0.3	47.4 ± 0.5
NDF insoluble	tr[b]	2.7 ± 0.2	tr[b]	43.7 ± 3.1	46.4 ± 2.7
NDF soluble	tr[b]	tr[b]	tr[b]	0.9 ± 0.0	0.9 ± 0.0
rat					
wheat bran					
feces	12.1 ± 0.1	13.3 ± 0.0	1.3 ± 0.0	16.0 ± 1.7	42.7 ± 2.3
NDF insoluble	12.2 ± 0.3	13.5 ± 0.5	0.8 ± 0.0	15.2 ± 0.4	41.7 ± 0.9
NDF soluble	tr[b]	tr[b]	tr[b]	0.7 ± 0.0	0.7 ± 0.0
cellulose					
feces	tr[b]	4.2 ± 0.1	0.8 ± 0.1	60.0 ± 2.7	65.0 ± 2.7
NDF insoluble	tr[b]	3.9 ± 0.4	tr[b]	52.8 ± 1.2	56.7 ± 1.1
NDF soluble	tr[b]	tr[b]	tr[b]	0.8 ± 0.1	0.8 ± 0.1

[a] Sugars are expressed as polysaccharides, i.e., x0.9. [b] Trace, <0.5%.

of sugar excreted daily (Slavin and Marlett, 1980; Balasubramanian, 1987; Balasubramanian et al., 1987; Lopez-Guisa et al., 1988). The fecal neutral sugar content of the human wheat bran feces was 7.9 g/day and of the human cellulose feces, 19.0 g/day. Daily neutral sugar intake derived from dietary fiber was approximately 30 g with the wheat bran diet (Johnson et al., 1988) and 24 g during the cellulose diet (Slavin et al., 1983). Comparison of daily total neutral sugar intake with excretion in the humans indicates that daily neutral sugar excretion with wheat bran supplemented diet was 26% of that consumed, and with the cellulose-supplemented diet, neutral sugar excretion was 79% of that consumed. The fecal neutral sugar content of the rat wheat bran feces was 1.4 g/day and of the cellulose feces, 0.9 g/day. In rats consuming about 20 g/day of diet, fiber intake from the cellulose diet was $\simeq$0.9 g/day and from the wheat bran diet, $\simeq$2.5 g/day. Therefore, all of the dietary fiber neutral sugars appeared in the feces of rats fed cellulose, and 56% appeared in the feces of rats fed wheat bran.

The apparent recovery of more dietary neutral sugars in the excreta of rats vs humans suggests more extensive fermentation of fiber in the human than in the rat colon. In each species these calculations also suggest a greater disappearance of wheat bran derived neutral sugars than of those from cellulose. This conclusion is consistent with evidence that wheat bran is more fermentable than purified cellulose both in rats (Balasubramian, 1987; Lopez-Guisa et al., 1988) and in man (Marlett and Johnson, 1985; Slavin and Marlett, 1980); however, such a conclusion assumes that all of the fecal neutral sugar is derived from unfermented dietary fiber. Endogenous mucin and bacteria contain polysaccharides, although their contribution to the total fecal neutral sugars is unknown. The use of the NDF method, which separates fecal polysaccharides into insoluble dietary fiber, i.e., NDF, and the NDF filtrate containing bacteria, mucin, and any unfermented but soluble dietary fiber would permit a better estimate of unfermented dietary fiber. More than 80% of the fecal neutral sugars from all samples were extracted into the NDF residue, although there were significant differences between the rat and human wheat bran samples (Table I); 81% of the fecal neutral sugars from the human wheat bran sample were recovered in the NDF, whereas 98% of the sugars in the rat wheat bran sample were recovered in the NDF. Thus, nearly 20% of the neutral sugars from the human wheat bran sample were solubilized by the neutral detergent solution. In both samples a larger proportion of the glucose than of either xylose or arabinose was extracted as NDF-soluble materials. Fecal NDF sugar content from the cellulose-containing samples differed from that in the wheat bran feces. Nearly all of the neutral sugars in the human cellulose feces, 98%, were recovered in the NDF, whereas only 87% of the fecal sugars were recovered in the NDF from rat cellulose excreta.

When the sum of the fecal neutral sugars in the NDF is used to calculate NDF digestibility, it was 80% in the humans consuming wheat bran, 28% in humans consuming cellulose, 52% in rats fed wheat bran, and 22% in rats fed cellulose. These results are in reasonable agreement with those we reported previously as NDF digestibilities. In previous studies we measured apparent NDF digestibility in humans during consumption of the wheat bran supplement with ad libitum diets as 68% (Marlett and Johnson, 1985) or during consumption of the purified cellulose supplement in conjunction with a constant mixed food diet as 23% (Slavin et al., 1981). In rats consuming wheat bran as part of an AIN 76A purified diet as the only fiber source, apparent NDF digestibility was 53%, and when cellulose was the fiber source, 22% (Balasubramanian, 1987). Nyman and co-workers (Nyman and Asp, 1982; Nyman et al., 1986) reported neutral sugar digestibility in the rat to be 54% and 41%, during ingestion of two different wheat brans, and 34% in humans ingesting wheat bran; these digestibilities were corrected for neutral sugar excretion during basal diets. The results from one of their rat studies is the same as what we determined, suggesting that either correction for basal neutral sugar excretion or use of NDF may provide an estimate of fiber digestibility. The differences in the human apparent wheat bran digestibilities between the two laboratories may be because different wheat brans were used.

The recovery of negligible amounts of xylose and arabinose in the fecal NDF filtrates from either species suggests that little of the wheat bran hemicellulose was incompletely fermented to soluble fiber components. It is generally agreed that by the time lumenal contents are excreted as feces most of the mucin has been fermented. Mucin is water soluble, and we have measured only 0.5–1.5% of the fecal neutral sugars in an aqueous extract (Cabotaje and Marlett, unpublished results). Thus, the carbohydrate in the NDF filtrate may serve as an index

Table II. Effect of Sodium Sulfite and Temperature on Human and Rat Fecal NDF Recovery and Composition[a]

	temp, °C	sodium sulfite	NDF % of feces	% of NDF	
				neutral sugars	total nitrogen
human feces					
wheat bran	100	+	23.2 ± 0.9[a]	65.0 ± 1.8	1.0 ± 0.1[a]
		−	25.6 ± 0.2[b]	61.0 ± 3.0	2.0 ± 0.1[b]
	40	+	23.7 ± 1.9[a]	65.3 ± 1.4	1.4 ± 0.0[c]
		−	26.4 ± 0.9[b]	64.8 ± 3.7	1.9 ± 0.0[b]
cellulose	100	+	47.6 ± 0.1[a]	91.1 ± 2.6[a]	1.4 ± 0.0[a]
		−	50.8 ± 1.2[b]	90.4 ± 2.6[a]	1.4 ± 0.0[a]
	40	+	53.6 ± 3.7[b]	86.4 ± 5.8[b]	1.3 ± 0.0[b]
		−	52.6 ± 1.2[b]	82.3 ± 0.7[b]	1.5 ± 0.0[c]
rat feces					
wheat bran	100	+	48.2 ± 0.2	78.4 ± 1.7	0.8 ± 0.0[a]
		−	48.8 ± 0.2	81.8 ± 3.2	0.9 ± 0.0[b]
	40	+	49.2 ± 0.5	79.7 ± 3.8	0.9 ± 0.0[b]
		−	50.2 ± 0.2	81.3 ± 3.7	1.1 ± 0.0[c]
cellulose	100	+	62.5 ± 0.4	82.6 ± 1.3	0.3 ± 0.0[a]
		−	63.1 ± 0.7	80.0 ± 1.8	0.5 ± 0.1[b]
	40	+	61.2 ± 1.2	76.8 ± 3.5	0.3 ± 0.0[a]
		−	63.2 ± 0.8	78.7 ± 1.5	0.4 ± 0.0[b]

[a] Numbers within each experimental group with different letters differ significantly, $p < 0.05$; neutral sugars are expressed as polysaccharides, i.e., $x0.9$.

of the bacteria present. Since bacteria contain more than carbohydrate, an estimate of fecal bacterial mass could be obtained by the calculation of 100 minus NDF weight. It has been reported that fermentation of wheat bran produces a greater bacterial mass (Stephen and Cummings, 1980), and the recovery of 8% of the human fecal total neutral sugars in the NDF filtrate is consistent with this evidence. The calculation of bacterial mass as 100 minus NDF yields a value of ~75%, which is higher than the 50% reported by Stephen and Cummings (1980). The fact that only 2% of fecal neutral sugars were solubilized from rat wheat bran feces by the detergent suggests a relativley smaller bacterial mass in rat wheat bran feces, a finding consistent with less apparent fermentation in the rat colon compared to the human large intestine. Because it has been shown that the cellulose was poorly fermented in humans, the recovery of little of the fecal sugars in the "bacterial" fraction would suggest no increase and perhaps even a decrease in the bacterial mass with ingestion of cellulose. The results from the rats are not in agreement with the human data. More of the fecal sugars were solubilized by the neutral detergent solution from the cellulose rat feces than from the wheat bran feces. A larger proportion of the glucose, however, was solubilized by the detergent in the wheat bran samples vs the cellulose samples, 12% vs 2% in the human samples and 4% vs 1% in the rat samples, respectively (Table I). This soluble glucose could reflect either partially fermented cellulose from the wheat bran or the cellulose fiber source or bacterial cell wall components. It is unlikely that the glucose reflects undigested starch; any starch escaping the small intestine is rapidly degraded by the microflora in both man and rats (Asp et al., 1987). Part of the reason for the differences between the species in the cellulose samples may be because different celluloses were used. The Solka Floc used in the human study was poorly fermented. When it was the sole fiber source in the diet, apparent NDF digestibility was 8% (Slavin et al., 1981), less than the digestibility measured in rats, 22% (Balasubramanian, 1987). Nyman et al. (1986) reported similar wheat bran fiber digestibilities in humans and rats. The wheat bran used in our human and rat studies was the same, i.e., AACC soft white wheat bran.

Sodium sulfite in the neutral detergent solution significantly decreased the fecal NDF yield from the human but not the rat samples when the analysis was conducted at 100 °C (Table II). At the lower temperature sodium sulfite reduced the NDF recovery from only the human wheat bran sample.

Sodium sulfite in the detergent solution significantly decreased the nitrogen remaining in the NDF from all samples except the human cellulose NDF obtained at 100 °C, although some of the differences were very small (Table II). Marlett and Johnson (1985) also reported lower fecal NDF and NDF nitrogen with the addition of sodium sulfite. It has been demonstrated previously that the browning reaction, assessed as nitrogen in NDF, does not occur at 40 °C (Robertson and Van Soest, 1981). Maillard product formation is a major concern in the drying of forages. In early work Van Soest (1965) demonstrated a doubling of the lignin content of forage if water was added during a 4-h 100 °C incubation. He proposed that the nitrogen in ADF can be an indicator of enzymatic browning and that this could be minimized by adding bisulfite (Van Soest, 1965). We hypothesized that the heat and moisture conditions in the NDF might permit the browning reaction to occur. In our study the interaction between sodium sulfite addition and temperature was not significant ($p > 0.1$), suggesting that there was no apparent Maillard product formation.

The neutral sugar content of the fecal NDFs varied with the sample (Table II). About two-thirds of the human wheat bran fecal NDF was neutral sugars, in contrast to 82–90% of the human cellulose fecal NDF. Rat fecal wheat bran and cellulose NDF were similar, about 75–80%, depending on the modification of the NDF method used (Table II). The use of sodium sulfite in the detergent solution had no effect on human fecal NDF neutral sugar yield. Temperature and sodium sulfite also had no effect on neutral sugar content of rat fecal NDF (Table II). Sodium sulfite was used in the NDF procedure because it attacks disulfide bridges. In subsequent experiments Robertson and Van Soest (1981) suggested that the sodium sulfite also may attack lignin and recommended that the sulfite be omitted from the NDF solution. Our results suggest that if the NDF analysis is to be gravimetric, the use of sulfite to remove nitrogen provides a more accurate measure of this fiber fraction.

Results of the measurements of fecal lignin are presented in Table III. Acid-insoluble residues or Klason lignin obtained from the hydrolysis of feces was always significantly higher than residues from the hydrolysis of

Table III. Measurements of Crude Lignin in Human and Rat Feces[a]

	% of fecal dry weight					
	fecal Klason lignin	ADF crude lignin	permanganate lignin	NDF crude lignin	temp, °C	sodium sulfite
human						
wheat bran	21.9 ± 0.5[a]	5.4 ± 0.3[b]	3.3 ± 0.3[c]	4.7 ± 0.4[b]	100	+
				6.0 ± 0.4[d]		−
				5.1 ± 0.7[b]	40	+
				6.2 ± 0.4[d]		−
cellulose	10.3 ± 0.1[a]	1.7 ± 0.1[b]	2.0 ± 0.5[bc]	1.5 ± 0.0[b]	100	+
				1.9 ± 0.3[bc]		−
				2.6 ± 0.5[cd]	40	+
				4.0 ± 1.9[d]		−
rat						
wheat bran	13.7 ± 0.0[a]	6.3 ± 0.1[b]	5.2 ± 0.6[c]	7.3 ± 0.5[d]	100	+
				10.1 ± 0.9[ef]		−
				8.8 ± 0.4[f]	40	+
				9.0 ± 0.4[f]		−
cellulose	3.9 ± 0.3[a]	0.9 ± 0.0[b]	1.9 ± 0.7[c]	1.6 ± 0.1[c]	100	+
				1.7 ± 0.5[c]		−
				1.8 ± 0.1[c]	40	+
				2.1 ± 0.7[c]		−

[a] Numbers within each experimental group with different letters differ significantly, $p < 0.05$.

the NDF, ADF crude lignin or permanganate lignin. The acid-insoluble residues after Saeman hydrolysis of NDF were lower when sodium sulfite was added and the analysis was conducted at 100 °C, probably a reflection of less nitrogen. Sodium sulfite produced a lower NDF crude lignin when the analysis was conducted at 40 °C only for the human wheat bran sample. Compared to the insoluble residue when NDF was obtained with sodium sulfite at 100 °C, the permanganate lignin values from the wheat bran, but not the cellulose samples, were lower (Table III). When comparisons are made to the ADF crude lignin, the NDF crude lignin recovery varied with the species, generally being higher in the rat and no different in the human samples.

One of the drawbacks to the use of the NDF analysis for feces was the inability to measure the neutral sugar content of the soluble fraction due to the presence of detergent and salts. Through the use of dialysis of a diluted aliquot, we removed the salts and a considerable amount of the detergent. Analysis of the NDF filtrate indicates that only a small portion of the fecal neutral sugars are solubilized by the detergent. The use of sodium sulfite had no effect on the neutral sugar content of fecal NDF but did lower the NDF gravimetric yield, suggesting that gravimetric fecal NDF determinations would be more accurate if sodium sulfite was used. The measure of lignin obtained by acid hydrolysis of these NDF residues was similar to the ADF crude lignin and always less than the Klason lignin. In our experience with food Klason lignin analysis, precipitated protein will be collected with Klason lignin if the residue is not neutralized (Marlett, 1990). Thus, we propose that NDF or ADF crude lignin would be a better estimate of fecal lignin than Klason lignin.

ACKNOWLEDGMENT

This research was supported by National Institutes of Health (NIH) Grant DK 21712 and the College of Agriculture and Life Sciences, University of Wisconsin—Madison. E.S.K. was a postdoctoral trainee funded by NIH Grant 5T32CA09451.

LITERATURE CITED

Asp, N.; Bjorck, I.; Holm, J.; Nyman, M.; Siljestrom, M. Enzyme resistant starch fractions and dietary fibers. *Scand. J. Gastroenterol., Suppl.* **1987**, *22* (*No. 129*), 29–32.

Balasubramanian, R. Calcium-Fiber Interactions in the Small and Large Bowel. Doctoral Dissertation, University of Wisconsin—Madison, 1987, 186 pp.

Balasubramanian, R.; Johnson, E. J.; Marlett, J. A. Effect of wheat bran on bowel function and fecal calcium in older adults. *J. Am. Coll. Nutr.* **1987**, *6*, 199–208.

Brauer, P. M.; Slavin, J. L.; Marlett, J. A. Apparent digestibility of neutral detergent fiber in elderly and young adults. *Am. J. Clin. Nutr.* **1981**, *34*, 1061–1070.

Englyst, H. N.; Cummings, J. H. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* **1984**, *109*, 937–942.

Goering, H. K.; Van Soest, P. J. *Forage fiber analysis apparatus, reagents, procedures and some applications*; Agricultural Handbook 379; U.S. Department of Agriculture: Washington, DC, 1970; pp 1–20.

Johnson, D. F.; Moore, W. E.; Zank, L. C. The spectrophotometric determination of lignin in small wood samples. *Tappi* **1961**, *44*, 793.

Johnson, E. J.; Roth, C. A.; Reinhardt, J. T.; Marlett, J. A. Dietary fiber intakes of nursing home residents and independent-living older adults. *Am. J. Clin. Nutr.* **1988**, *48*, 159–164.

Lopez-Guisa, J. M.; Harned, M. C.; Dubielzig, R.; Rao, S. C.; Marlett, J. A. Processed oat hulls as potential dietary fiber sources in rats. *J. Nutr.* **1988**, *118*, 953–962.

Marlett, J. A. Analysis of dietary fiber in human foods. In *Dietary Fiber Chemistry, Physiology and Health Effects*; Kritchevsky, D., Ed.; Plenum Publishing: New York, 1990; pp 31–48.

Marlett, J. A.; Chesters, J. G. Measuring dietary fiber in human foods. *J. Food Sci.* **1985**, *50*, 410–414, 423.

Marlett, J. A.; Johnson, E. J. Composition of fecal fiber from human subjects. *J. Nutr.* **1985**, *115*, 650–660.

Neilson, M. J.; Marlett, J. A. A Comparison between detergent and nondetergent analyses of dietary fiber in human foodstuffs, using high-performance liquid chromatography to measure neutral sugar composition. *J. Agric. Food Chem.* **1983**, *31*, 1342–1347.

Nyman, M.; Asp, N. Fermentation of dietary fibre components in the rat intestinal tract. *Br. J. Nutr.* **1982**, *47*, 357–366.

Nyman, M.; Asp, N.; Cummings, J.; Wiggins, H. Fermentation of dietary fibre in the intestinal tract: comparison between man and rat. *Br. J. Nutr.* **1986**, *55*, 487–496.

Ray, A. A., Ed. *SAS Users Guide: Statistics*; SAS Institute: Cary, NC, 1982.

Robertson, J. B.; Van Soest, P. J. The detergent system and its application to human foods. In *The Analysis of Dietary Fiber in Food*; James, W. P. T., Theander, O., Eds.; Dekker: New York, 1981; pp 122–158.

Slavin, J. L.; Marlett, J. A. Influence of refined cellulose on human bowel function and calcium and magnesium balance. *Am. J. Clin. Nutr.* **1980**, *33*, 1932–1939.

Slavin, J. L.; Brauer, P. M.; Marlett, J. A. Neutral detergent fiber, hemicellulose and cellulose digestibility in human subjects. *J. Nutr.* **1981**, *111*, 287–297.

Slavin, J. L.; Marlett, J. A.; Neilson, M. J. Determination and apparent digestibility of neutral detergent fiber monosaccharides in women. *J. Nutr.* **1983**, *113*, 2353–2359.

Southgate, D. T. Use of the Southgate method for unavailable carbohydrates in the measurement of dietary fiber. In *The Analysis of Dietary Fiber in Food*; James, W. P. T., Theander, O., Eds.; Dekker: New York, 1981.

Stephen, A. M.; Cummings, J. H. The microbial contribution to human faecal mass. *J. Med. Microbiol.* **1980**, *13*, 45–56.

Theander, O.; Westerlund, E. Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. *J. Agric. Food Chem.* **1986**, *34*, 330–336.

Theander, O.; Aman, P.; Miksche, L. E.; Uasuda, S. Carbohydrates, polyphenols and lignin in seed hulls of different colors from turnip rape seed. *J. Agric. Food Chem.* **1977**, *25*, 270.

Van Soest, P. J. Use of detergents in analysis of fibrous feeds. III. Study of effects of heating and drying on yield of fiber and lignin in forages. *J. Assoc. Off. Anal. Chem.* **1965**, *48*, 785–790.

Van Soest, P. J.; Wine, R. J. Use of detergents in the analysis of fibrous feed IV. Determination of plant cell-wall constituents. *J. Assoc. Off. Anal. Chem.* **1967**, *50*, 50–55.

Received for review July 5, 1989. Revised manuscript received January 16, 1990. Accepted February 1, 1990.

Registry No. Lignin, 9005-53-2; arabinose, 10323-20-3; xylose, 58-86-6; galactose, 59-23-4; glucose, 50-99-7; sodium sulfite, 7757-83-7; nitrogen, 7727-37-9; cellulose, 9004-34-6.

Analysis for Daminozide in Apple Juice by Anion-Exchange Chromatography–Particle Beam Mass Spectrometry

In Suk Kim, Fassil I. Sasinos, Robert D. Stephens, and Mark A. Brown*

Hazardous Materials Laboratory, California Department of Health Services, 2151 Berkeley Way, Berkeley, California 94704

Daminozide [Alar, succinic acid mono(2,2-dimethylhydrazide)] is measured directly as the parent compound in 11 commercially obtained apple juice samples at levels ranging from 0 to 280 ppb (average 160 ppb). Sample preparation involves only evaporation and treatment with methanol and acetone to precipitate sugars and inorganic salts. Analysis is via anion-exchange chromatography particle beam mass spectrometry, with positive chemical ionization using isobutane as a reagent gas. A method detection limit of 25 ppb is achieved in part by a signal enhancement resulting from the constant addition of 0.4 mM malic acid to the mobile phase. Malic acid also modifies the nature of the mass spectrometer response to daminozide, changing it from a nearly cubic to a linear relationship and also changing relative ion intensities, between the base ion of M + 1 − H_2O without and M + 1 with 0.4 mM malic acid.

The use of daminozide [Alar, succinic acid mono(2,2-dimethylhydrazide)] as a plant growth regulator has generated controversy because of its reported animal carcinogenicity (Toth et al., 1977; National Cancer Institute, 1978) and because of a recent suggestion that children may be at risk from eating apples treated with this compound (Natural Resources Defense Council, 1989). Average daminozide residues in apples, applesauce, and apple juice have been reported to range from 4.9 ppm (Environmental Protection Agency, 1985) to 0.48 ppm (Conditt et al., 1988) and will presumably decline after the manufacturer withdraws the compound from the market (Thayer, 1989).

The highly hydrophilic nature of daminozide (solubility, grams/100 g of solvent: water 10 g; methanol, 5 g; acetone, 2.5 g; acetonitrile, 0.2 g; xylene, insoluble (Environmental Protection Agency, 1985)) suggests that conventional liquid/liquid based extraction procedures and gas chromatography based methods will not be appropriate for direct analysis of this compound. Existing analytical methods detect daminozide only directly via alkaline hydrolysis of the parent compound to the easily oxidized 1,1-dimethylhydrazine (UDMH) followed by extraction and derivatization to yield a stable, usually volatile adduct that can be easily analyzed by gas chromatography. Differences in these methods are only in the means of detection of the adduct, utilizing colorimetry (Edgerton et al., 1967), electron capture (Newsome, 1980), and mass spectrometry (Conditt et al., 1988). Typical detection limits are from 0.1 to 2 ppm.

Although risk and exposure assessments of daminozide emphasize its hydrolysis product UDMH, both the succinyl and *N*-methyl moieties of the intact parent compound bind covalently to both liver DNA and protein of treated mice and to human hemoglobin in vitro via oxidation with hydrogen peroxide (Brown and Casida,

0021-8561/90/1438-1223$02.50/0