# Analysis of Fiber Components in Feeds and Forages Using Gas-Liquid Chromatography

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Procedures are described for study of fiber components of feeds and forages. Fifteen feeds and forages were delignified with sodium chlorite and hydrolyzed with trifluoroacetic acid. The hydrolyzed sugars are derivatized to their corresponding alditol acetates and analyzed on the gas chromatograph. Comparisons between the detergent fiber analysis system and the proposed gas chromatograph system are reported. Electron microscopy was used to evaluate chemical action of neutral detergent, acid detergent, sodium chlorite, ammonium oxalate, and trifluoroacetic acid.

Originally, fiber in feeds and forages was analyzed by the crude fiber method purported to Einhof in 1806, and the ramifications of its use have been discussed by Van Soest and McQueen (1973). Salo (1965) has reviewed other carbohydrate analyses in feeds and feces and reported two approaches to the analysis of fiber in the literature: (1) fiber determined by gravimetric techniques after digestion in a solution that would rid the cell wall of cytoplasm and (2) fractionation of the cell wall into its various components of cellulose, hemicellulose, and lignin.

In the 1960's, Van Soest and others developed methods which isolated cell wall components with neutral and acid detergent (Van Soest, 1963; Van Soest and Wine, 1967). These techniques were also used to determine cellulose, hemicellulose, and lignin (Goering and Singleton, 1971; Morrison, 1973; Van Soest, 1975). Southgate (1969a,b) designed a fractionation scheme to estimate vegetable fiber in materials commonly used in human nutrition, which is more finite, but more time consuming.

Many physical and metabolic effects have been attributed to fiber in the diet (Kelsay, 1978); however few reports have dealt with the actions of fiber components such as mineral binding due to the exchange properties of fiber or possible absorption of metabolites through the amorphous nature of the fibrous components (Froix, 1976). Due to difficulties in defining dietary fiber's mode of action and inadequate understanding of fiber components, many reports have attempted to rename fiber.

Recently, derivatization of sugars to their corresponding alditol acetates has made it possible to analyze carbohydrates quantitatively using gas chromatography (Gumner et al., 1961; Sawardeker et al., 1965). Albersheim et al. (1967) have published a series of papers using gas chromatography to analyze the cell walls of sycamore cells (Talmadge et al., 1973; Baver et al., 1973). Sloneker (1971) also attempted to use the alditol acetate derivatization technique to determine cellulose and hemicellulose in plant tissues which contain lignin.

The objective of this study was to examine and modify the techniques of Albersheim et al. (1967) to delignified feeds and forages (Collings et al., 1978) and to observe plant cell wall ultrastructure after digestion and hydrolysis with chemical treatments using scanning electron microscopy.

## EXPERIMENTAL SECTION

**Preparation of Plant Samples.** Sixteen feeds and forages were chosen to represent a broad cross-section of substrates (Table I). Each was hand collected, dried at 60 °C for 48 h, ground in a Wiley mill (1-mm screen), and stored in glass bottles. A second dry matter (A) on each sample was performed after grinding to account for water absorption (Froix, 1976; Collings et al., 1978). A 0.50–1.00-g sample was predigested in 200 mL of 0.5% w/v ammonium oxalate and delignified with sodium chlorite as described by Collings et al. (1978).

Table I. Feeds and Forages

| common name | scientific name |
|---|---|
| Kentucky bluegrass | *Poa pratensis* |
| reed canarygrass | *Phalaris arundinacea* |
| full bloom | |
| second cutting | |
| Orchardgrass | *Dactylis glomerata* |
| bromegrass | *Bromis inermis* |
| tall fescue | *Festuca arundinacea* |
| reed foxtail | *Alopecurus pratensis* |
| red clover | *Trifolium pratense* |
| ladino clover | *Trifolium repens* |
| elodea | *Elodea canadensis* |
| birdsfoot trefoil | *Lotus corniculatus* |
| wheat straw | *Triticum aestiuum* |
| wheat bran | |
| wheat middlings | |
| cattle manure fiber | |

[a] Washed manure from animals fed corn silage ration.

**Preparation of Alditol Acetates.** A 50–60 mg delignified sample (F) was transferred to a 18 × 150 mm heavy duty Wheaton tube containing 10 mL of 1 N trifluoroacetic acid. Myoinositol (5–7 mg) (G) was added to each tube as an interval standard. Each delignified sample was run in triplicate. The tubes were sealed with slotted stoppers and aluminum seals and hydrolyzed at 120 °C for 60 min in an autoclave. After the hydrolysis, the samples were filtered through a preweighed sintered glass crucible (no. 3). The filtrate was then collected and poured into a 50-mL round-bottom flask. Total uronic acids were determined by colorimetric analysis of the ammonium oxalate and trifluoroacetic acid filtrates (Blumendrantz and Asboe-Hansen, 1973). The residue was washed with 50 mL of deionized distilled water and dried for 24 h. The filtrate was evaporated to dryness under a stream of filtered air in a 60 °C water bath. The hydrolyzed hemicellulose sugars in the filtrate were reduced to their respective alditols with sodium borohydride (1 g) in 1 N ammonia (50 mL) for 1 h with occasional swirling. The reduction was ceased with glacial acetic acid until the reaction stopped. Ten milliliters of methanol was then added and evaporated to dryness under a stream of filtered air in a 60 °C water bath. Five more 10-mL methanol additions were added (Albersheim et al., 1967) and

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Table II. Analysis of Feeds and Forages

| substrate | NDF | AOF | ADF | hemicellulose (NDF-ADF) | hemicellulose (alditol acetate) | cellulose permanganate | cellulose TFAA | lignin (permanganate) | lignin (sodium chlorite) |
|---|---|---|---|---|---|---|---|---|---|
| Kentucky bluegrass | 59.90[a] | 73.58[b] | 37.60 | 22.30 | 18.94 | 28.88[a] | 37.55[b] | 5.56[a] | 6.06[a] |
| reed canarygrass | | | | | | | | | |
| full bloom | 59.61[a] | 66.01[b] | 34.74 | 24.87 | 14.96 | 27.98[a] | 26.88[a] | 5.85[a] | 5.35[a] |
| second cutting | 58.24[a] | 68.38[b] | 34.87 | 23.37 | 16.54 | 24.71[a] | 28.37[b] | 5.33[a] | 4.94[a] |
| orchardgrass | 57.36[a] | 70.06[b] | 38.70 | 18.66 | 14.36 | 30.10[a] | 29.96[a] | 4.68[a] | 6.73[a] |
| bromegrass | 60.12[a] | 69.81[b] | 38.38 | 21.74 | 15.31 | 28.14[a] | 27.35[a] | 5.54[b] | 5.54[a] |
| tall fescue | 62.44[a] | 71.59[b] | 38.50 | 23.94 | 24.50 | 29.88[a] | 29.96[a] | 3.13[b] | 7.29[a] |
| reed foxtail | 57.15[a] | 69.27[b] | 33.81 | 23.34 | 17.72 | 25.92[a] | 29.49[b] | 4.46[b] | 4.99[a] |
| red clover | 29.62[a] | 56.88[b] | 21.48 | 8.14 | 6.65 | 16.43[a] | 20.26[a] | 3.80[b] | 8.02[a] |
| ladino clover | 29.77[a] | 56.05[b] | 25.81 | 3.96 | 12.17 | 20.03[a] | 19.68[a] | 4.94[a] | 5.95[a] |
| elodea | 31.16[a] | 60.66[b] | 22.67 | 8.49 | 10.34 | 17.97[a] | 21.21[a] | 4.09[b] | 3.65[a] |
| birdsfoot trefoil | 50.09[a] | 65.45[b] | 37.70 | 12.39 | 15.44 | 28.94[a] | 28.79[a] | 8.72[b] | 12.44[a] |
| wheat straw | 79.84[a] | 84.14[b] | 51.75 | 28.09 | 19.62 | 34.10[a] | 47.37[b] | 12.44[b] | 8.72[a] |
| wheat bran | 52.25[a] | 60.39[b] | 13.95 | 38.30 | 12.79 | 9.93[a] | 15.77[b] | 4.08[b] | 7.73[a] |
| wheat middlings | 49.77[a] | 64.81[b] | 14.66 | 35.11 | 32.91 | 10.19[a] | 10.36[a] | 4.63[b] | 7.09[a] |
| manure fiber | 83.10[a] | 91.36[b] | 51.35 | 31.75 | 11.15 | 40.04[a] | 46.63[b] | 9.61 | 13.07[a] |

[a,b] Values with different superscripts are significantly different ($P < 0.05$). Comparisons made between NDF and AOF, permanganate TFAA cellulose and permanganate and sodium chlorite lignin. [c] Washed manure from corn-silage fed Hereford steer.

evaporated to dryness as before. Acetic anhydride (4 mL) was then added and the round-bottom flask was sealed with a Wheaton Scientific blueprint no. 07350, Wheaton Scientific, Millville, NJ, rubber stopper and wired down. The mixture was then heated at 120 °C for 1 h in an autoclave.

**GLC Quantification of Alditol Acetates.** A 3–5-μL sample of the autoclaved mixture was injected into a gas chromatograph (Varian Model 1800 aerograph) equipped with a hydrogen flame ionization detector. A stainless steel column (120 × 0.3 cm) packed with 0.2% polyethylene glycol adipate, 0.2% polyethylene glycol succinate, and 0.4% silicone XF-1150 on Gas-Chrom P (100–120 mesh) was used. Other GLC parameters were as follows: column temperature, programmed between 135–200 °C with a 10-min holding at 135 °C after injection of the sample, followed by 1 °C/min increase in temperature; helium flow rate of 30 mL/min, injection temperature of 210 °C, detector temperature of 250 °C, attenuation of 32× and range of 1 mV.

**Calculation of Results.** The following formula was used to calculate the percent hemicellulosic sugars and cellulose:

$$\% \text{ cellulose or } \% \text{ sugar} = \frac{C \times \dfrac{\text{wt of sugar or cellulose}}{BC/D}}{\text{sample wt} \times A} \times 100$$

$$\text{wt of cellulose in } E = JE/F \qquad \text{wt of sugar in } E = GIE/FH$$

where $A$, dry matter of dry ground sample; $B$, oxalate fiber residue scrappings; $C$, weight of oxalate fiber at 0 min; $D$, weight of oxalate fiber at 30 min; $E$, weight of holocellulose; $F$, weight of holocellulose sample (50 mg); G, weight of myoinositol; $H$, myoinositol GLC peak area; $I$, sugar GLC peak area; $J$, weight of cellulose.

**Trifluoroacetic Acid Hydrolysis.** To determine the optimum hydrolysis time as specified by Albersheim et al. (1967), samples of Reed Canarygrass (full bloom) delignified tissue (holocellulose) was prepared. Fifty-milligram samples plus 5–7 mg of myoinositol were hydrolyzed in triplicate in 10 mL of 1 N trifluoroacetic for 15, 30, 45, 60, 75, and 90 min.

**Methanol Additions.** As shown by Albersheim et al. (1967), five additions of methanol appeared to be sufficient to permit methylation of the hemicellulose sugars in sycamore cells. To test this in whole plants, samples of Reed Canarygrass (full bloom) delignified tissue was prepared. Fifty milligram, samples plus 5–7 mg of myoinositol were hydrolyzed in 10 mL of 1 N trifluoroacetic acid for 60 min. After collection of the filtrate, evaporation, and reduction, one–six 10-mL additions of methanol were tested.

**Electron Microscopy.** Dried (60 °C) wheat straw was ground through a Wiley mill (1-mm screen) and treated with neutral or acid detergent (Goering and Van Soest, 1970), ammonium oxalate or sodium chlorite (Collings et al., 1978), and trifluoroacetic acid. Each sample was adhered to aluminum stubs using double-stick tape, coated with 200–300 A gold in a film-vac sputter coater, and observed in an ISI-Super III SEM operated at 15 kV.

## RESULTS AND DISCUSSION

The study of plant cell wall components is of relevance not only to a basic understanding of structural integrity, but also to a more concise assessment of nutrient availability. A number of researchers have expressed the opinion that a more definitive chemical nomenclature is needed to better interpret the metabolic role of fiber in nutrition (Trowell, 1972; Spiller and Amen, 1975; Spiller et al., 1976). To this end, the evaluation of new approaches to the analysis of fiber should continue to be investigated.

Typical values for fibrous components in the 16 feeds and forages as determined by neutral detergent (NDF), acid detergent (ADF), potassium permanganate cellulose, and lignin are presented in Table II. These methods are relatively easy to perform and the values obtained describe the general physical structure of the various feeds and forages. Neutral detergent fiber represents the plant cell walls minus pectins (Bailey and Ulyatt, 1970), some insoluble ash (Van Soest, 1975), and possibly some arabinose (Barton, 1977). Acid detergent fiber includes the cellulose, lignin, and insoluble ash (Goering and Van Soest, 1970). Recently, ADF has been shown to contain hemicellulose sugars (Bailey and Ulyatt, 1970; Collings and Yokoyama, 1978). The difference between the two detergent fiber fractions is an estimate of hemicellulose which can be either overestimated or underestimated depending on the substrate (Van Soest, 1975). Oxidation of lignin in the acid

Table III. Hemicellulose Components Determined by Alditol Acetates

| | hemicellulose components[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | hemicellulose[a] | glucose | galactose | arabinose | xylose | mannose | rhamnose | uronic acids | xylose arabinose ratio |
| Kentucky bluegrass | 18.94 | 8.34 | 5.20 | 14.65 | 58.60 | | | 13.22 | 4.00 |
| reed canarygrass | | | | | | | | | |
| full bloom | 14.96 | 8.46 | 4.50 | 14.42 | 62.16 | | | 11.13 | 4.31 |
| second cutting | 16.54 | 9.21 | 6.25 | 16.52 | 58.96 | | | 9.68 | 3.57 |
| orchardgrass | 14.36 | 10.26 | 3.33 | 13.07 | 54.43 | | | 18.91 | 4.16 |
| bromegrass | 15.31 | 11.21 | 4.66 | 15.77 | 56.95 | | | 11.41 | 3.61 |
| tall fescue | 24.50 | 5.65 | 3.92 | 15.22 | 63.47 | | | 10.21 | 4.17 |
| reed foxtail | 17.72 | 12.20 | 6.69 | 17.59 | 49.75 | | 1.68 | 12.09 | 2.83 |
| red clover | 6.65 | 28.33 | 3.36 | 6.15 | 6.91 | 2.94 | | 68.62 | 1.12 |
| ladino clover | 12.17 | 10.23 | 9.91 | 7.90 | 20.33 | 4.26 | 3.18 | 43.51 | 2.57 |
| elodea | 10.34 | 23.24 | 7.37 | 5.00 | 10.20 | 3.17 | 1.60 | 49.17 | 2.04 |
| birdsfoot trefoil | 15.44 | 20.82 | 7.88 | 8.23 | 36.84 | 3.06 | 2.01 | 21.00 | 4.48 |
| wheat straw | 19.62 | 10.26 | 2.72 | 11.18 | 67.99 | | | 7.89 | 6.08 |
| wheat bran | 12.79 | 28.33 | 3.36 | 18.41 | 29.06 | 1.29 | | 19.55 | 1.58 |
| wheat middlings | 32.91 | 25.18 | 1.81 | 27.90 | 42.62 | 1.24 | | 1.86 | 1.53 |
| cattle manure fiber | 11.15 | 11.86 | 2.74 | 6.38 | 74.05 | 2.74 | | 4.97 | 11.61 |

[a] Hemicellulose expressed as a percent DM. [b] Sugars expressed as a percent of hemicellulose.

detergent fiber with potassium permanganate and ashing at 493 °C will yield values for lignin, cellulose, and insoluble ash.

Determination of fiber in feeds and forages involves the disruption of the plant cell walls to rid the plant of cytoplasm by digestion with neutral detergent (Goering and Van Soest, 1970) or with ammonium oxalate (Siddiqui and Wood, 1974; Collings et al., 1978). Upon comparison of the neutral detergent fiber and ammonium oxalate fiber (AOF) values (Table II) for the 16 feeds and forages, all AOF values were significantly higher ($P < 0.05$) than their corresponding NDF values. Marked differences were observed in ladino clover and red clover values. These differences in cell wall values for the substrates may have been due to losses in pectins and uronic acids (Bailey and Ulyatt, 1970), insoluble ash (Van Soest, 1975), possible losses of arabinose (Barton, 1977), or might represent differences in protein and ash content. These differences will be elucidated in further research.

After delignification of the cell wall with sodium chlorite (Collings et al., 1978), each substrate was hydrolyzed in trifluoroacetic acid (TFAA). The percent cellulose in each substrate can be determined after collecting the residue from TFAA hydrolysis. A comparison of permanganate cellulose and TFAA cellulose (Table II) yielded similar results in most substrates except Kentucky bluegrass, Reed canarygrass (second cutting), Reed foxtail, wheat bran, and wheat straw which have significantly higher cellulose values as determined by TFAA hydrolysis. A comparison of permanganate lignin and sodium chlorite lignin of the 15 substrates has shown that in general sodium chlorite lignin tended to be higher in value (Collings et al., 1978).

Hydrolysis of the delignified tissue with trifluoroacetic acid and derivatization to the corresponding alditol acetate yields a pattern of the hemicellulose sugars which include glucose, galactose, mannose, xylose, arabinose, rhamnose, and possible ribose. In these calculations, uronic acids, which may include those from pectins, were included in the hemicellulose. Uronic acids are also found as a component of the cell wall (Buchala and Wilkie, 1973). Xylose, arabinose, galactose, and glucose were apparent in all substrates examined (Table III). Xylose was the highest in concentration in all substrates with the exceptions of red clover, ladino clover, and elodea. Uronic acids were the predominant hemicellulosic component in these substrates. Mannose was found in red clover, ladino clover, elodea, birdsfoot trefoil, wheat bran, wheat middlings, and cattle manure fiber in small amounts but not in the other substrates. Rhamnose did not appear to be a common hemicellulosic component; however, it was 31.8% of the ladino clover hemicellulose and lesser amounts were detected in reed foxtail, elodea, and birdsfoot trefoil hemicellulose. The xylose/arabinose ratio has been used as an indicator of plant maturity, i.e., the ratio increases with age of the plant (Buchala and Wilkie, 1973). The ratio ranged from 2.83 to 4.31 in the grasses, whereas the other substrates showed more variable amounts of xylose and arabinose as indicated by a ratio of less than 2.00 as seen in red clover, wheat bran, and wheat middlings and a ratio greater than 6.00 as seen in wheat straw and cattle manure fiber. The similarity in xylose and arabinose content and in the xylose/arabinose ratio in grasses indicates possible structural similarities not apparent in the other substrates.

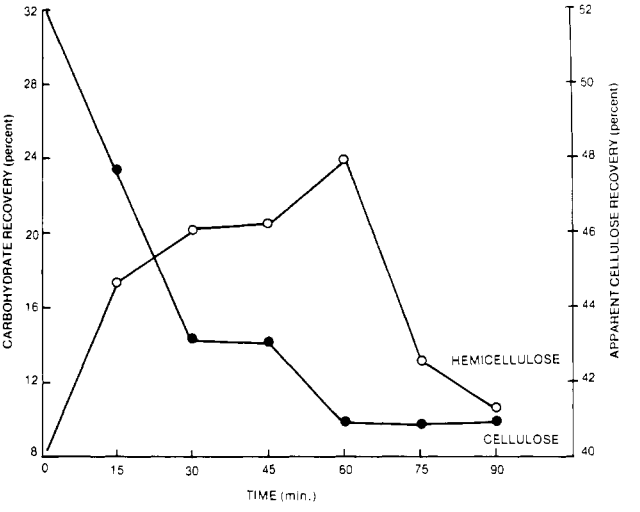


**Figure 1.** Length of trifluoroacetic acid hydrolysis time on hemicellulose and cellulose recovery.

**Hydrolysis Time and Derivatization.** A 60-min trifluoroacetic acid hydrolysis was used in previous studies of sycamore cells (Albersheim et al., 1967). Six hydrolysis times were tested to examine if the length of hydrolysis affected yield of hemicellulose and cellulose (Figure 1). The concentration of cellulose decreased steadily up to 60 min of hydrolysis at which time cellulose concentration remained constant. A hydrolysis time of 60 min also showed the maximum yield of hemicellulose and decreased

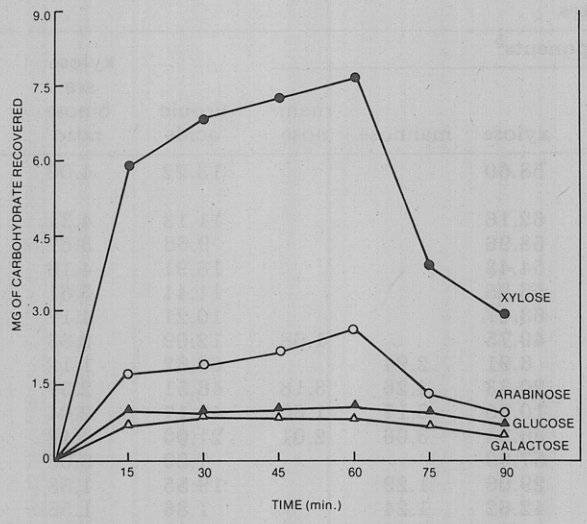


**Figure 2.** Length of hydrolysis time and sugar yield.

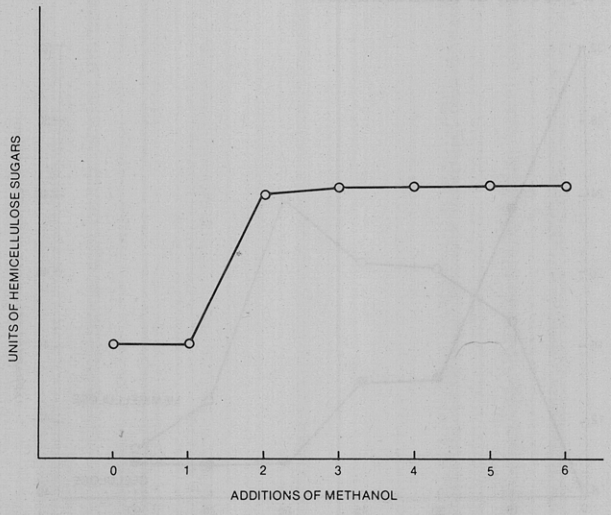


**Figure 3.** Methanol additions.

with a hydrolysis time greater than 60 min which is in agreement with Albersheim et al. (1967). An examination of hemicellulosic sugars in Reed canarygrass (Figure 2) paralleled the response of hemicellulose (Figure 1). Both arabinose and xylose were degraded at hydrolysis times greater than 60 min. These results are in agreement with Albersheim et al. (1967) in sycamore cells.

Albersheim et al. (1967) showed that five methanol additions were necessary in order to get maximum derivatization of hemicellulosic hydrolysate. In agreement with this finding, maximum hemicellulosic sugar yield was apparent at two methanol additions (Figure 3) in our study; however, three–five methanol additions could be used to insure complete derivatization of various samples with no deleterious effects.

**Electron Microscopy.** Several investigators have indicated possible losses and complications in the detergent fiber system (Bailey and Ulyatt, 1970; Porter and Singleton, 1971; Morrison, 1973; Van Soest, 1975; Barton, 1977). Scanning electron microscopy was used in order to examine the effects of neutral detergent, acid detergent, ammonium oxalate, trifluoroacetic acid, and sodium chlorite treatment of cell walls of wheat straw. Both neutral detergent and ammonium oxalate have been described to rid the plant cell wall of cell cytoplasm and debris (Figure 4a). Neutral detergent (a solution of sodium lauryl sulfate, disodium dihydrogen ethylenediaminetetraacetate dihydrate, disodium hydrogen phosphate, sodium borate decahydrate, and ethylene glycol) appeared to open the plant cells of wheat straw as was seen in Figure 4b. Small frequent disruptions (arrows) were seen in the cell wall (Figure 4b) which may be due to the possible losses of uronic acids (Ulyatt and Bailey, 1970), insoluble ash (Van Soest, 1975), or arabinose (Barton, 1977). The cell wall of ammonium oxalate treated wheat straw (Figure 4c,d) appeared to have opened cell walls (arrows) with typical flaking of the cell wall, but no apparent losses. Both neutral detergent and ammonium oxalate appeared to rid the cell wall of debris as shown in Figure 4a.

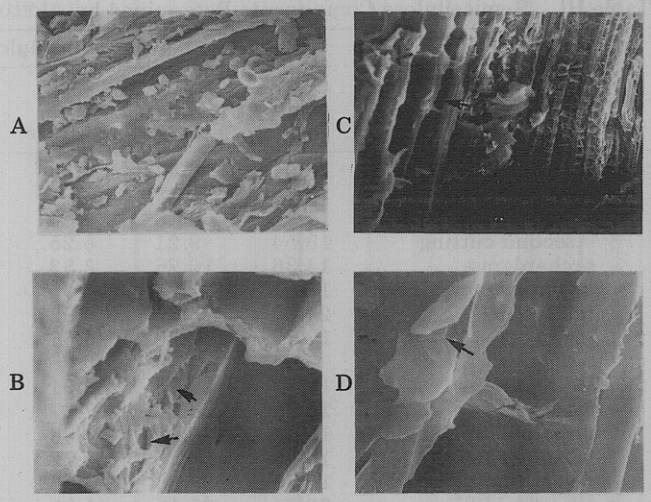


**Figure 4.** Effect of neutral detergent and ammonium oxalate upon wheat straw. (A) Control sample: surface is coated with debris; no open cells apparent; ×1000. (B) Section treated with neutral detergent. Open cells are quite apparent. The wall is intact with small disruptions evident (arrows). X3000. (C) Section treated with ammonium oxalate. Open cells are quite apparent. X400. (D) Section treated with ammonium oxalate. Cell wall is smooth and glossy with small typical flaking (arrows). X3000.

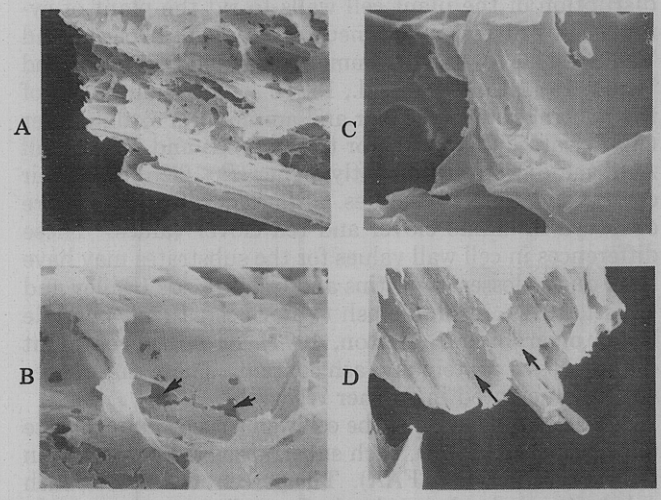


**Figure 5.** Effect of acid detergent, trifluoroacetic acid and sodium chlorite upon wheat straw. (A) Section treated with acid detergent. X1000. (B) Section treated with acid detergent. Ruptured cell wall are apparent (arrows). X3000. (C) Section treated with sodium chlorite in dilute acid. Cell wall very much intact. X10 000. (D) Section treated with trifluoroacetic acid. Cellulose sheets clearly evident (arrows). X1000.

The structure of hemicellulose in many plants has been examined extensively (Talmadge et al., 1973; Baver et al., 1973) and illustrated as cross-linked chains of sugars evenly distributed over a core of cellulose sheets. Acid detergent has been reported to remove the hemicellulose, leaving cellulose, lignin, and some ash. The chemical mode of

attack as seen in Figure 5a–b appeared to be very localized (arrows) and may not remove all the hemicellulose.

The action of sodium chlorite is to oxidize the lignin of a plant cell wall without extracting carbohydrates from cellulose and hemicellulose. Figure 5c shows the cell wall of sodium chlorite delignified wheat straw. The cell wall showed no disruptions and appeared to be evenly extracted. After trifluoroacetic acid hydrolysis, cellulose is collected in a crucible, dried, and weighed. Figure 5d illustrates wheat straw TFAA with typical cellulose sheets (arrows).

The use of detergents in analyzing for fiber in feeds and forages offered quick, easy, and repeatable methodology; however, possible losses and complications have been described. The chemical action of acid detergent appears to be unsatisfactory for yielding a fraction of only cellulose as the major carbohydrate component; however, it does provide a repeatable measure of fiber in animal rations. The alditol acetate derivatization system for feeds and forages can be used to analyze for cellulose, hemicellulose, hemicellulose sugars, and uronic acids and lignin and offers additional structural information about the fiber components in the plant cell wall.

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# Sample Preparation and the Determination of Lead in Chewing Gum by Nonflame Atomic Absorption Spectrometry

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A procedure for the dissolution of chewing gum by combined wet and dry ashing is described. Lead concentrations in ten varieties of chewing gum are determined by the heated graphite tube atomizer technique of atomic absorption spectrometry.

Recent chemical literature reveals an increasing interest in the determination of trace concentrations of essential and nonessential elements in foods and other agricultural products. The elucidation of the exact roles played by these elements in various biological systems is far from complete. The advances of the past decade in the development of extremely sensitive, yet relatively inexpensive, instrumentation such as the electrothermal atomizers in atomic absorption spectrometry have made trace-level analysis widely accessible (Syty, 1974). A particularly useful and thorough review of the methods and techniques used in the determination of metals in foods has been published recently (Crosby, 1977).

An extensive search of the analytical literature has yielded no published methods for the determination of heavy metals in chewing gum. Apparently, this material has not been examined by either atomic absorption or by other modern spectrometric methods of analysis. It became, therefore, the purpose of this study to develop a method for the determination of trace concentrations of lead in gum by atomic absorption with electrothermal atomization. Lead was selected as the first element to be tested because of its health hazard to children at dietary amounts above 300 μg/day (King, 1971).

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