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MODIFYING WOOD TO INCREASE ITS *IN VITRO* DIGESTIBILITY [1,2,3,4]

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WOOD residues, of 70 to 80% carbohydate, are a potential source of dietary energy for ruminants. However, only a minor percentage of this carbohydrate can be utilized by rumen microflora without some form of pretreatment (Scott, Millett and Hajny, 1969). The high lignin content and the low cell contents (Van Soest, 1964, 1967) are believed to be the controlling factors.

Delignification (Hvidsten, 1940; Edin, Helleday and Nordfeldt, 1941; Saarinen, Jensen and Alhojärvi, 1958) and saccharification (Forest Products Laboratory, 1960, 1964) provide highly digestible products, but they are too costly. Irradiation with high-energy electrons (Lawton et at., 1951), subdivision into micron-size particles (Virtanen and Koistenen, 1944; Virtanen and Nikkilä, 1946; Pew, 1957; Pew and Weyna, 1962; Stranks, 1959; Dehority and Johnson, 1961), and steeping in dilute alkali (Saarinen *et al.*, 1958; Stranks, 1961; Wilson and Pigden, 1964) provided enhanced utilization of wood or forage carbohydrate by bacteria, enzymes, or ruminants.

This investigation was undertaken to more fully define optimum conditions of treatment and to establish the relative effectiveness of various treatments to increase the in vitro rumen digestibility of wood-residue carbohydrate. Treatments evaluated were the following: Irradiating with high-energy electrons, vibratory ball milling, swelling in liquid ammonia, and swelling in alkali solution.

Methods and Materials

The *in vitro* rumen procedure for estimating digestibility of the various native and modified wood substrates has been developed by the authors. It is a modification of the first stage of the Tilley and Terry method (1963). Modifications include using a 1:1 ratio of rumen fluid to McDougal's buffer, a 0.25-g sample, a capped glass incubation vessel, an extended incubation period with the tubes maintained in a horizontal position with periodic agitation, and having a total liquid volume of 38 milliliters. Cotton linters and alfalfa were used as reference substrates. All samples were ground to pass 40 mesh prior to treatment. Data shown are the average of duplicate samples. The earlier experiments utilized a 48-hr. fermentation period, whereas those conducted later were for 96 to 144 hr. Extensive use of the procedure has shown it to yield an average relative standard deviation of about 2%, with a range from 0.5 to 5.0%.

In the electron-irradiation experiments, 1/16-in. (1.59 mm) Sitka spruce veneer and 1/4-in. (6.35 mm)-thick aspen boards were irradiated at the facilities of the Midwest Irradiation Center, Rockford, Ill., at dosage levels of 10^6, 10^7, 5×10^7 and 10^8 rep. The electron beam was generated with an 8-mev. linear accelerator rather than with the resonant transformer used in the earlier study by Lawton *et al.* (1951). The irradiated spruce was initially broken down with a guillotine and the irradiated aspen with a jointer, and then were ground in a Wiley mill to pass 40 mesh.

Vibratory ball milling was done in the Forest Products Laboratory mill (Pew, 1957) in a 4 C cold room to prevent heating. A sample charge of 5 g of ovendry wood ground to pass 40 mesh was used. The mill was charged with about 4 kg of 1/4-in. (6.35 mm) polished and hardened steel balls. Milling times ranged from 0 to 240 minutes. The balls and the

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[4] Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed by the U. S. Department of Agriculture.
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TABLE 1. *IN VITRO* DRY-MATTER DIGESTIBILITY OF VARIOUS WOODS AND THE BARKS

Substrate	Digestibility[a] Wood	Bark
	%	%
Hardwoods		
Red alder	2	..
Trembling aspen	33	30
Trembling aspen (ground-wood fiber)	37	"*
Bigtooth aspen	31	..
Black ash	17	45
American basswood	5	25
Yellow birch	6	16
White birch	8	..
Eastern cottonwood	4	..
American elm	8	27
Sweetgum	2	..
Shagbark hickory	5	..
Soft maple	20	
Soft maple buds	36	..
Soft maple small twigs	37	..
Sugar maple	7	14
Red oak	3	..
White oak	4	..
Softwoods		
Douglas-fir	5	..
Western hemlock	0	..
Western larch	3	7
Lodgepole pine	0	..
Ponderosa pine	4	..
Slash pine	0	..
Redwood	3	..
Sitka spruce	1	..
White spruce	0	..

[a] 96-hr. incubation. For comparison: 96-hr. digestibility of cotton linters was 90%; of a reference alfalfa. 61%.

ground product were separated by mechanical agitation on a screened pan.

The ammoniation treatments were conducted in two ways. In the first, the substrates were steeped in anhydrous liquid ammonia either in an insulated open beaker at —33 C or in a pressure vessel at temperatures of 30, 60 and 90C for various periods of time. Internal pressures were approximately 155 p.s.i. at 30 C, 360 p.s.i. at 60 C and 725 p.s.i. at 90C. In the second, the substrates were exposed to anhydrous ammonia gas at 30C, and at tank pressure of about 155 p.s.i. for various periods of time. After treatment, the samples were removed from the treating vessels, spread in a thin layer to permit evaporation of excess ammonia and then vacuum-dried overnight at room temperature.

Alkali treatment consisted of immersing the substrates in 1% sodium hydroxide, using 100 ml of solution for 5 g of sawdust. This provided a solution-to-wood ratio of 20:1 and an NaOH-to-wood ratio of 1:5. Following a 1-hr. soaking, the residues were washed to neutrality with water, air-dried, and assayed by the *in vitro* procedure using a 5-day incubation.

The barks of the various species had substantially higher digestibilities than their corresponding woods. To determine the proportion of loss of total dry matter caused by nonnutritive solubles rather than by true digestion, duplicate 0.25-g bark samples were soaked with 38 ml of H_2O for 4 days at 39 C in the same manner as was used in the *in vitro* procedure. A few drops of toluene were added to each tube to prevent mold or bacterial attack. The contents of each tube were filtered through a Gooch filter crucible; the residue was washed with a 35-ml portion of H_2O, and the total filtrate collected directly in a 100-ml volumetric flask. After making up to volume, aliquots of the filtrate were analyzed for total reducing substances, and results reported as weight of glucose. An additional portion of the filtrate was acidified to 4% H_2SO_4 and given a standard secondary hydrolysis (1 hr. at 120C) to break down oligosaccharides and phenolic glycosides (Saeman *et al.*, 1954).

Results and Discussion

Assay of Various Woods and Their Barks. A summary of the *in vitro* digestibility of various woods and their barks is given in table 1. Although all of the hardwood species examined showed some degree of digestibility, they exhibited a rather broad range of values —from a low of 2% to a high of approximately 35%. Aspen was most digestible, and it was followed by soft maple and black ash. The softwoods were not digested significantly. These results are in general agreement with those of Stranks (1959) obtained with a pure culture of *Ruminococcus flavefaciens.* He reported that aspen and ash yielded substantial *in vitro* digestibilities, whereas elm, birch, and basswood required pre-treatment (alkali and chlorite) before they were appreciably digested. Nehring and Schramm (1951a, b) also reported that ash, aspen, maple and elm were superior roughage sources for sheep compared to oak and birch.

There was a rough parallelism between the degree of digestibility of the bark and the wood of a given species, with the bark yielding higher digestibility values (table 1). With the disposal of bark becoming an increasing problem, the possibility of utilizing it as a cattle feed deserves further investigation.

Most barks contain appreciable quantities of soluble fatty substances that may be readily digested. A typical proximate analysis of air-dry aspen bark, for example, showed the following results: Protein, 2.3%; ether extract. 9.5%; fiber, 36.1%; ash, 3.7%; and moisture, 6.6%. The ether extract constitutes almost 10% of aspen bark. Twigs and leaves are also more digestible than the stem wood. because they contain less lignin and have a higher percentage of digestible protein (Nehring and Schütte, 1951) and ether extract. The soft maple buds and twigs had digestibility coefficients of about 35% compared to 20% for the wood.

Table 2 shows the water solubility of various hardwood barks and the digestibility obtained by the *in vitro* technique. Significant amounts of hydrolyzable oligosaccharides or of phenolic glycosides are present only in the extracts of aspen and black ash barks. Approximately, one-half of the total weight of material in a hardwood-bark water extract appears to be carbohydrate, nutritionally useful to the ruminant. It is assumed the remaining portion of the material consists primarily of phenolic compounds. The weight of bark solubilized by water alone is about one-half the weight of bark solubilized during the *in vitro* rumen assay. It might he concluded that approximately three-fourths of the *in vitro* digestibility for a hardwood bark is carbohydrate, and about one-fourth is noncarbohydrate.

High-Energy Electron Irradiation. The effect of exposure to increasing levels of electron irradiation on the *in vitro* digestibility of aspen and spruce is shown in table 3. Aspen digestion is essentially quantitative if it is assumed that only carbohydrate has been solubilized at an electron dosage of 10^8 rep. However, the lignin content of this aspen was 19.5%, and it might be expected that some lignin-degradation products would be formed at this dosage level. If water-soluble, these would contribute to the figure for dry-matter digestibility. In any event, electron irradation is an effective means for enhancing the digestibility of aspen. It does very little for spruce, however; the maximum digestibility was only 14% at the highest dosage level. Although higher dosage levels would probably further improve digestibility, they would also increase the level of carbohydrate destruction. From earlier work on the use of electron irradiation to enhance wood saccharification (Saeman, Millett and Lawton, 1952), it was shown that carbohydrate destruction was about 15% at 10^8 rep. and increased to about 4.5% at 5×10^8 rep. The product of the latter dosage was almost completely water soluble and was strongly acidic.

TABLE 2. WATER SOLUBILITY OF VARIOUS HARDWOOD BARKS AND EXTENT OF CARBOHYDRATE DISSOLUTION

Species	Weight loss	Carbohydrates in extract: Free sugars	Carbohydrates in extract: Sugars after hydrolysis[a]	Dry-matter digestibility[b]
	%	%	%	%
Aspen	16.0	3.3	6.2	30
Black ash	22.1	6.5	10.0	45
Yellow birch	11.5	5.0	4.8	16
American elm	10.4	5.0	3.6	27
Sugar maple	5.5	1.7	1.9	14

[a] 1 hr. at 121 C with 4% H_2SO_4; neutralized with lime.
[b] 96-hr. rumen-fluid digestibility of unextracted bark.

TABLE 3. EFFECT OF ELECTRON IRRADIATION ON THE DRY-MATTER DIGESTIBILITY OF ASPEN AND SPRUCE

Radiation dosage	Digestibility[a]: Aspen[b]	Digestibility[a]: Spruce
rep.[c]	%	%
0	55	3
10^6	52	3
10^7	59	5
5×10^7	70	8
10^8	78	14

[a] 5-day *in vitro* rumen-fluid assay.
[b] *Populus tremuloides.* This sample was from a board containing a high proportion of tension wood fibers. Tension wood is characterized by an exceptionally high carbohydrate-to-lignin ratio; thus, the high untreated digestibility of this aspen sample in comparison with that shown in table 1.
[c] Roentgen equivalent physical.

Although electron irradiation appears to provide an effective means for enhancing the carbohydrate digestibility of certain woods, irradiation costs are too high for the process to be of interest commercially. It was recently estimated that the treatment cost was about $150 per ton at the dosage level of 10^8 rep. (Anonymous, 1959)

Vibratory Ball Milling. The effect of vibratory ball milling on the *in vitro* digestibility of aspen and red oak is shown graphically in figure 1. *In vitro* digestibilities of both woods increased rapidly with milling time to about 30 min., anti then they increased more slowly with further milling. A strong digestibility dependence on time of inclubation was also evident; at least 5 days of incubation were required for digestibilities to attain 90% or more of their plateau values.

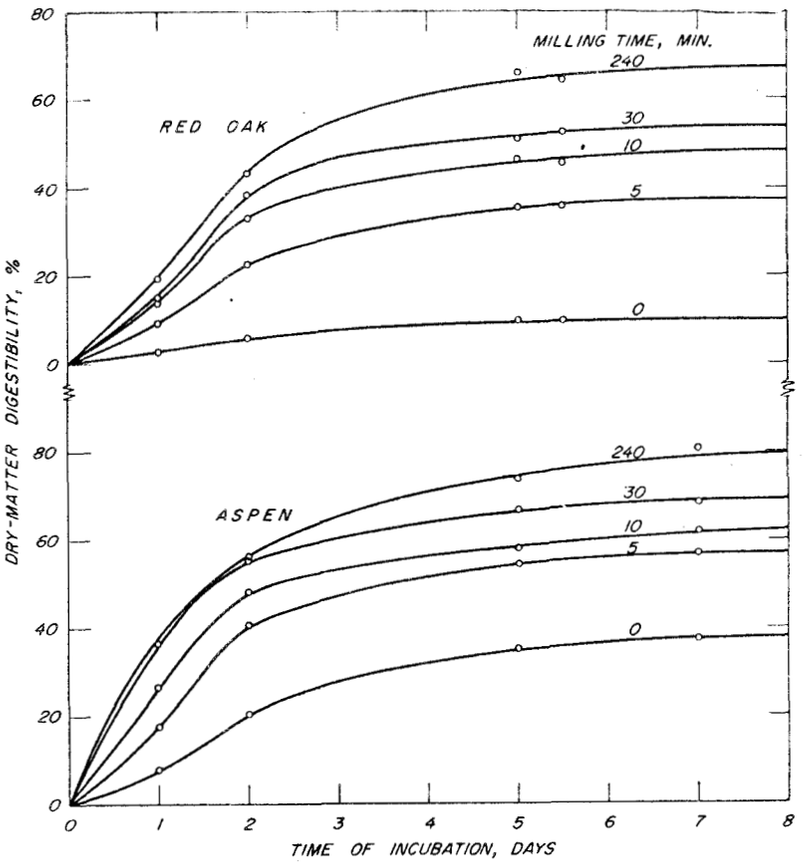

Figure 1. Relation of dry-matter digestibility of red oak and aspen to time of incubation and extent of virbratory ball milling.

In vitro digestibility of aspen and red oak which had been milled for 240 min. was 80% and 67%, respectively. That this was not merely a solubilization effect was demonstrated by the results of an enzymatic hydrolysis of the milled products using a high-potency cellulase (Onozuka SS, 1,500 μ/g).[7] Sugar production rather than weight loss was the measured variable in this case. The 240-min.-milled aspen and oak produced 63% and 57% of their weight as glucose, respectively. Sugar production from the unmilled aspen and oak was 10.0% and 0.0%, respectively. Of the total carbohydrates in aspen and red oak, 70 to 80% were made accessible to cellulase digestion by vibratory ball milling.

Water-solubility measurements were made on each of the milled samples using the techniques described earlier for barks. The results in table 4 show that the quantity of water solubles increased progressively with increased milling time, with oak maintaining about 2% higher solubility than aspen at all milling times. Only small amounts of free sugar were present in the extracts. After dilute acid hydrolysis, however, about 50% of the total solubles were present as free sugars, indicative that about one-half of the water-extracted solids was carbohydrate and of probable nutritive value to the ruminant. Subtraction of the valves for noncarbohydrate solids from the maximum *in vitro* digestibilities attained by aspen and oak after 240 min. of milling yielded carbohydrate digestibilities of about 75% for aspen and 60% for oak. These values are in reasonable agreement with reducing sugar values obtained by enzyme assay.

In figure 2 *in vitro* rumen digestibility is plotted as a function of milling time for five hardwood species. The digestibility values

[7] Kanematsu-Gosho-USA-Inc., 1 Whitehall Street, New York N. Y. 10004.

TABLE 4. EFFECT OF VIBRATORY BALL MILLING ON WATER-SOLUBLE EXTRACTIVES OF ASPEN AND RED OAK WOOD

Species	Time of milling	Total solubles by weight loss	Reducing sugars in extract (as glucose)	
			Before hydrolysis	After hydrolysis
	Min.	%	%	%
Aspen	0	1.8	0.3	0.7
	5	2.6	0.3	1.3
	10	3.2	0.4	1.3
	30	4.1	0.4	2.4
	60	6.4	0.5	3.7
	120	9.7	0.6	6.1
	240	13.7	0.8	8.9
Red oak	0	4.1	1.5	1.0
	5	5.4	1.4	1.8
	10	6.0	1.4	2.0
	30	6.7	1.4	2.4
	60	8.4	1.6	3.9
	120	11.2	1.6	5.9
	240	15.3	1.9	8.7

are those obtained with 5-day incubation. The first 20 to 30 min. of milling appear to have the major influence on digestibility. A digestibility plateau is apparently attained beyond which additional milling is of no value.

From the curves in figure 2, it is evident that at least 140 min. of vibratory milling are required to approach the digestibility plateau. However, a digestibility equivalent to that of the alfalfa control (60% with 96-hr. incubation) was achieved with only 10 to 15 min. of milling for aspen and sweetgum and about 80 min. for red oak. It appears doubtful that hickory or alder could attain this level with any degree of milling. Softwoods were even less responsive to vibratory milling, since the maximum digestibility was 18% for five different softwoods (Douglas-fir, western hemlock, ponderosa pine, slash pine and redwood) that were milled for 120 minutes.

It is difficult to ascribe definite reasons for the wide variation in response between hardwoods and softwoods or even for the variation exhibited within the hardwood group. Certainly particle size alone is not the governing factor. All wood samples received the same degree of milling, and settling tests in water indicated similar particle-size distribution. The controlling factor must be the quantity, chemical nature, and distribution within the cell walls of lignin.

The very selective response of the various species to vibratory ball milling makes this technique of limited value as a general means for upgrading the digestibility of wood residues. Moreover, there is a question whether finely ground wood will function as effectively in the ruminant as it does in *in vitro* assay. With forages, fine grinding has increased the *in vitro* digestibility of cellulose, but it has not produced similar responses when fed to ruminants when digestibility in fact has been

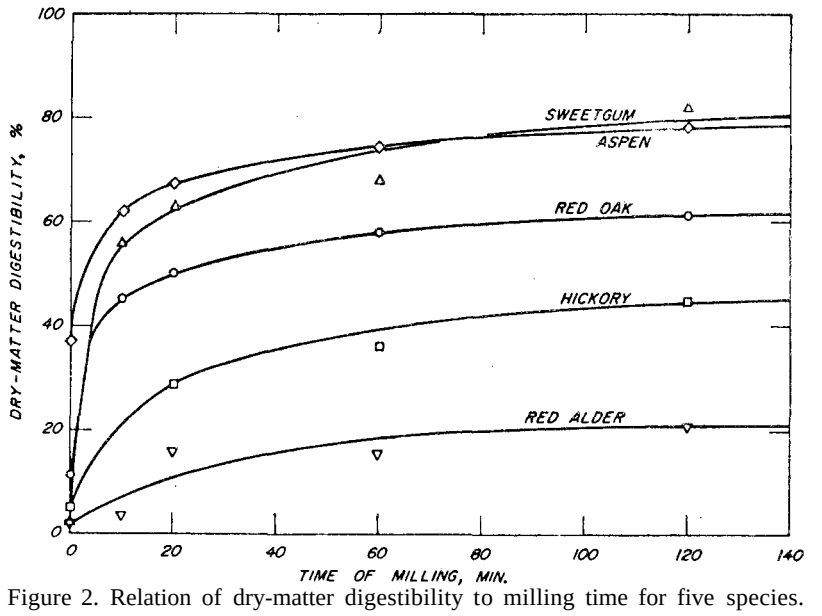

Figure 2. Relation of dry-matter digestibility to milling time for five species.

TABLE 5 IN VITRO DIGESTIBILITY OF ASPEN SAWDUST EXPOSED TO ANHYDROUS LIQUID AND GASEOUS AMMONIA

Treatment[a]		
Chemical	Time	Digestibility[b]
	Hr.	%
Control	..	33
Liquid NH_3	1	51
Gaseous NH_3	½	48
	1	44
	2½	47
	4	45
	8½	51
	16	46
	40	50
	73	46
Average	..	47

[a] At 30 C.
[b] 5-day incubation.

decreased (Rodrigue and Allen, 1960; Moore, 1964; Meyer, Kromann and Garrett, 1965).

Treatment with Anhydrous Liquid Ammonia. As shown in table 5, treatment of aspen sawdust with anhydrous liquid or gaseous ammonia provided a substantial increase in *in vitro* digestibility, raising it to approxmately that of a good hay. There is no significant difference in the digestibilities between the two types of treatment. The effect is rapid; a 1/2-hr. treatment with gaseous ammonia at 30 C yielded the same digestibility value as a 73-hr. treatment. Only at atmospheric pressure, where the boiling temperature is —33 C, was a time effect observed. Here, maximum digestibility required somewhat more than a 1-hr. treatment.

Dr. Caulfield of the Forest Products Laboratory staff showed on the basis of X-ray diffraction measurements that, although treatment with liquid ammonia caused a phase change from cellulose I to cellulose III (Segel, Loeb and Creely, 1954), the total crystalline content was probably not altered appreciably. Since digestibility of aspen wood was increased more than 50% with liquid ammonia treatment, support is given the idea that the pertinent action of the treatment is the ammonolysis of cross links of glucuronic acid esters (Tarkow and Feist, 1968).

Hardwoods which have been treated with liquid ammonia and air-dried have a markedly increased swelling capacity in water (Tarkow and Feist. 1968). This swelling action provides greater access to the structural carbohydrates by rumen bacteria and their associated enzymes. An additional nutritive benefit is the increased nitrogen content of the ammoniated product through formation of amides and ammonium salts by reaction with the acetyl and uronic acid ester groups of the wood. Kjeldahl analysis of ammoniated aspen showed 9% crude "protein" compared to a control value of 0.5%. Aspen appears to be unique in its digestibility response to ammoniation. The digestibilities of ammonia-treated spruce and red oak were 2% and 7 to 10%, respectively, *In vivo* verification of the aspen results is in progress. Additional work is needed to more closely define the responses of the various species.

Treatment with Aqueous Sodium Hydroxide. The results of a 5-day *in vitro* digestion show a range of response to the alkali treatment for the various species investigated (table 6).

Aspen and basswood, attaining a digestibility of 55%, are outstanding in their response to alkali pre-treatment The tenfold increase for basswood is especially intriguing. Bigtooth aspen is only slightly less digestible than trembling aspen. Black ash, white birch, and soft maple show an intermediate response with digestibilities ranging between 35% and 40%. The other species show digestibilities of less than 20%. Douglas-fir and Sitka spruce, which are softwoods with a maximum *in vitro* digestibility of 1% and 2%, respectively, did not respond to the alkali treatment.

To better define conditions for optimum processing, aspen was treated at room temperature with 0.5% and 1.0% solutions of sodium hydroxide at various liquid-to-solid ratios. Then it was washed to neutrality, dried and assayed. The results in table 7 show that from 5 to 6 g of NaOH per 100 g of wood are necessary for a maxinium effect on

TABLE 6. EFFECT OF ALKALI TREATMENT ON THE DRY-MATTER DIGESTIBILITY OF VARIOUS HARDWOODS

		5-day digestibility	
Species	Yield	Control	Treated[a]
	%	%	%
Trembling aspen	87	33	55
Bigtooth aspen	90	31	49
Black ash	91	17	36
American basswood	89	5	55
White birch	92	8	38
Yellow birch	94	6	19
Eastern cottonwood	93	4	11
American elm	93	8	14
Soft maple	92	20	41
Red oak	94	3	14
White oak	90	4	20

[a] 5-g wood treated for 1 hr. with 100 ml 1% NaOH, washed to neutrality and dried.

in vitro digestibility. This was attained with a 12:1 liquor-to-wood ratio at the 0.5% alkali level or a 6:1 ratio with 1% alkali. It is interesting that the minimum quantity of sodium hydroxide needed for attaining maximum digestibility is roughly equivalent stoichiometrically to the combined acetyl and carboxyl content of the aspen. This supports our contention (Tarkow and Feist, 1969) that the main consequence of alkali treatment is the breaking (by saponification) of intermolecular ester bonds. Rupture of these cross-links promotes the swelling of wood in water beyond normal water-swollen dimensions; thus it favors increased enzymatic and microbiological penetration into the fine structure of wood. At the optimum conditions (6 g NaOH to 100 g wood) the yield is about 95%. The 5% loss in weight is caused by saponification and removal of acetyl groups during the water wash. Animal-feeding trials with alkali-treated aspen are under may.

When the alkali used exceeds about 6%, the yields drop below 95% because of the ready solubility of wood hemicelluloses in alkali solutions. The problem could be eliminated by using the "dry" alkali treatment developed by Wilson and Pigden (1964) for use with wheat straw. At a 6:100 NaOH-to-wheat straw ratio, *in vitro* and *in vivo* dry-matter digestibilities of the treated straw averaged about 57% in contrast to about 41% for the untreated straw. *In vitro* digestibility of poplar wood was increased from 5% to 50% on treatment with a 9% "dry" alkali (based on the wood).

TABLE 7. EFFECT OF ALKALI TREATMENT VARIABLES ON THE *IN VITRO* DRY-MATTER DIGESTIBILITY OF ASPEN

NaOH concentration	Solution to wood	NaOH per 100 g of wood	Treating time	Yield	Digestibility[a]
%		G	Hr.	%	%
0	0	0	0	100	37
0.5	4:1	2	2	98	47
	8:1	4	2	98	50
	12:1	6	2	95	55
	16:1	8	2	93	53
1.0	2:1	2	1	98	41
	4:1	4	1	96	48
	6:1	6	1	95	51
	8:1	8	1	94	50
	10:1	10	1	93	54
	20:1	20	1	87	50

[a] 6-day incubation.

Summary and Conclusions

Among the 24 wood species investigated, three species exhibited the highest *in vitro* digestibilities—32% for aspen, 17% for black ash, and 20% for soft maple. Values for the remaining species ranged from 0 to 8%. Barks with the added nutritional potential of their fatty constituents were generally more digestible than their corresponding woods.

Four pre-treatments were investigated for their effectiveness in increasing the *in vitro* digestibility of carbohydrates from wood residues. All were effective to varying degrees, but the results were quite species dependent. Hardwoods were generally more responsive to treatment than softwoods. Although electron irradiation increased spruce digestibility from 0 to 14%, it increased the digestibility of aspen from 32% to more than 75%. Vibratory ball milling was very effective with aspen, sweetgum, and red oak, yielding carbohydrate digestibilities approaching those of the feed grains (70 to 80%; Morrison, 1956). Anhydrous liquid ammonia increased the digestibility of aspen wood more than 50%, but it was relatively ineffective on red oak and Sitka spruce. Treatment with 1% sodium hydroxide solutions raised the *in vitro* digestibility of a number of hardwood species ts equal the digestibilities of medium-quality hay. Animal-feeding trials with ammonia- and alkali-treated aspen are in progress.

From a rough estimate of overall costs, the four wood-modification techniques may be listed in the following ascending order: Swelling in aqueous sodium hydroxide, swelling in liquid ammonia, vibratory ball milling, and irradiating by high-energy electrons.

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