甘蔗バガスの加圧・ NaOH 処理による化学組成・消化率への影響 : II. セルロース調製への影響(畜産学科)

The Effects of Treatment of Bagasse with Sodium Hydroxide Under Steam Pressure on Chemical Changes and Digestibility

II. Effect on Cellulose Preparation

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I INTRODUCTION

In the experiment reported previously,[7] Hawaiian bagasse obtained from variety 50-7209 consisting approximately 54% of sugarcane grown in the state was treated with sodium hydroxide (0, 5, and 10% by weight of bagasse) under steam pressure (atmospheric, 150 and 300 PSI) for 5, 15 and 25 minutes in order to determine their effects on sample recovery, dry matter content and proximate nutrient contents of the material. As a result it was found: 1) Treated bagasse recovery was greatly affected by pressure and NaOH showing the destructing effect of the factors on bagasse structure. 2) Main effect of pressure and NaOH were significant in all proximate nutrients studied. 3) The correlations of sample recovery with proximate nutrients revealed that soluble substances or those readily altered by pressure and NaOH were lost in the decant solution and the residue or recovered bagasse material became high in crude fiber content.

This experiment was conducted, using the same bagasse materials treated under the same conditions as those in the previous experiment, to evaluate the effect of pressure, NaOH and time on the parameters such as alcohol extraction, delignification, holocellulose content, hemicellulose content and cellulose content involved in the process of bagasse cellulose preparation.

II MATERIALS AND METHODS

The bagasse material used and experimental design employed were the same as those in previous study.[7]

Cellulose was prepared applying the method employed by Tu.[9] The ground samples were first extracted with 95% ethanol using gold-fish ether extractor instead of Soxhlet apparatus for 48 hours. The substances soluble in the solvent were designated as alcohol extracts. Then, the material was delignified with sodium chlorite at pH 4. The delignified product, holocellulose, was then extracted with 10 % potasium hydroxide solution under nitrogen overnight to separate cellulose. The mixture was filtered and washed with 10% acetic acid solution and water until it became neutral. The substance disappeared was considered to be hemicellulose. The residue was dried and weighed as oagasse cellulose.

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All data were subjected to the analysis of variance procedures for 3 x 3 x 3 randomized factorial design.[8] When differences were observed, Duncan's Multiple Range Test was applied to determine the significance of differences between treatment means.

III RESULTS AND DISCUSSION

The analysis of variance for alcohol extraction, delignification and contents of holocellulose, hemicellulose and cellulose, and summarized effects of NaOH and pressure on these parameters are presented in Tables 1 and 2, respectively.

Table 1. Analysis of variance for alcohol extraction, delignification, holocellulose, cellulose and hemicellulose (loss in KOH solution)

Source of variance	Degree of freedom	Alcohol extraction	Delignified lignin	Holocel-lulose	Cellulose	Loss in KOH solution
Replication (3)	1					
NaOH (3:N)	2	7.5510	11.9684	27.7784	26.4402	124.4462**
Pressure (3:P)	2	636.2706**	153.1224**	1125.2204**	460.8271**	290.9086**
Time (3:T)	2	38.3080*	1.3939	28.7970	18.9247	145.8406**
N×P	4	174.3563**	61.4258**	265.7515**	23.2501*	322.9751
N×T	4	7.5445	3.1963	3.1173	15.2822	10.4485
P×T	4	7.7306	6.7457	20.4473	5.0527	37.0629
N×P×T	8	6.2854	13.4189	28.5413	2.6789	33.3340
Error	26	9.2606	9.8835	14.8273	8.3133	19.2570

* P<.05 ** P<.01

Alcohol extractives (waxes, fats, essential and fixed oils, oleoresin, sterols, organic acids, vitamins, protenaceous substances, etc.) were affected by pressure (P<.01) and time (P<.05), but not by NaOH. The extractives increased (P<.05) from 9.20% to 13.20% and 20.91% as pressure was increased from atmospheric to 150 and 300 PSI, respectively. Fifteen minutes (15.57%) and 25 minute (15.03%) had higher (P<.05) values than 5 minutes (12.81%), although these results are not presented in the table. Interaction (P<.01) between NaOH and pressure was observed. At atmospheric pressure, addition of NaOH increased the amount, although at both levels of pressure it was deccreased by use of NaOH except the 10% NaOH at 150 PSI treatment. Increase in alcohol extractives, together with decrease in sample recovery which was caused by decrease in crude protein and N.F.E., indicates increase in chemical or physical changes in fiber system. This relation is explained by significantly (P<.01, r = -0.7641) high negative correlation of alcohol extractives with sample recovery. Guggolz et al.[3] reported that treatment of bagasse with 4% NaOH (w/w) at 400 PSI for 40 seconds decreased amount of doubly extracted residue (consisting of Soxhlet extraction with a benzen alcohol mixtrue followed by a 4-hour and 16-hour, 85°C extraction with 0.5% ammonium oxalate solution) from 83% to 64%. Treatment with steam at 400 PSI for 80 seconds decreased the value from 83% to 58%. Kamstra[4] also found that treatment of bagasse with steam at

Table 2. Summarized effects of NaOH and pressure on the amounts(%) of alcohol extract (AE), delignification(DL), holocellulose(HLCE), cellulose(CE) and hemicellulose (HCE) and interaction of these factors

NaOH (%)	Product	Pressure (PSI) 0	150	300	Mean
0	AE	4.09*	10.66	12.84	9.20c
	DL	4.75	4.32	8.99	6.01b
	HLCE	91.20	85.01	78.17	84.79a
	CE	45.67	45.28	42.47	44.81c
	HCE	46.03	39.73	34.02	39.92a
5	AE	13.30	11.54	15.32	13.32b
	DL	16.00	9.99	9.23	11.74a
	HLCE	70.97	78.00	75.45	74.81b
	CE	49.72	48.18	51.85	49.91b
	HCE	21.26	30.29	26.19	25.91b
10	AE	27.56	18.97	16.21	20.91a
	DL	9.64	11.41	8.57	9.87a
	HLCE	62.86	69.48	75.20	69.18c
	CE	57.88	52.53	54.36	54.93a
	HCE	4.91	17.92	20.83	14.55c
Mean	AE	14.89	13.72	14.79	
	DL	10.13	8.57	8.93	
	HLCE	75.01	77.50	76.26	
	CE	51.09	48.66	48.89	
	HCE	24.07b	29.31a	27.01ab	

a, b, Values with same superscript within the same criterion are not significantly ($P > .05$) different.
*Average of 6 samples.

400 PSI increased the benzen alcohol extraction from 0% to 28%. In these two workers' data the increase in extractives were accompanied with increased digestibility (from 40% to 70%). Knapp et al.[5] found 3.5% to 4.7% benzen alcohol extractives for untreated bagasse.

The means of 27 treatment combinations for alcohol extractives as well as other parameters examined in the process of cellulose preparation and their comparison are shown in Table 3. Treatment with 0% NaOH under 300 PSI for 15 minutes (No. 8, 29.74%) gave the highest value which was not different from treatments 9 (26.97%) and 7 (25.97%), but different ($P<.05$) from the rest of treatment combinations including controls; 1 (4.00%), 3 (4.09%) and 2 (4.19%).

Delignification was changed ($P<.01$) by pressure, but not by NaOH and duration of treatment. A very complicated interaction of NaOH × pressure was found. At 150 PSI, 5% NaOH decreased delignification from 16.00% to 9.99%, whereas at 300 PSI it increased from 9.64% to 11.41%. At 0% NaOH delignification decreased from 16.00% to 9.64% as pressure was increased from 150 to 300 PSI, while at the 5% NaOH treatment it increased from 9.99% to 11.41%. At atomospheric pressure, increase in NaOH from 5% to 10% improved delignification from 4.32% to 8.99%, although at 150 and 300 PSI it was decreased from 9.99% to 9.23% and from 11.41% to 8.75%, respectively.

Table 3. Effects of NaOH, pressure and time on cellulose preparation (%)**

NaOH (%)	Pressure (PSI)	Time (min.)	No.*	Alcohol extraction	Deligni-fication	Holocel-lulose	Cellulose	Loss in KOH
		5	1	4.00^{i}	6.12^{hijk}	89.99^{a}	44.02^{ij}	45.87^{ab}
	0	15	2	4.19^{i}	8.92^{k}	91.85^{a}	45.50^{ghij}	47.96^{a}
		25	3	4.09^{i}	4.16^{k}	91.75^{a}	$47.49^{defghij}$	44.27^{bc}
		5	4	7.35^{hi}	13.21^{bc}	79.44^{bcde}	45.43^{ghij}	34.01^{ef}
0	150	15	5	16.68^{cdef}	18.88^{a}	64.55^{hij}	$49.43^{bcdefghi}$	15.02^{j}
		25	6	15.07^{cdefg}	15.90^{ab}	69.04^{fghij}	54.29^{abcd}	14.75^{j}
		5	7	25.97^{ab}	12.73^{bcde}	61.30^{j}	55.30^{ab}	6.01^{k}
	300	15	8	29.74^{a}	5.57^{ghijk}	64.69^{hij}	59.19^{a}	5.51^{k}
		25	9	26.97^{ab}	10.64^{cdef}	62.60^{ij}	59.16^{a}	3.23^{k}
		5	10	9.47^{fghi}	3.53^{k}	86.96^{ab}	44.50^{hij}	42.47^{c}
	0	15	11	11.09^{efghi}	4.58^{jk}	84.34^{abc}	46.18^{fghij}	38.16^{d}
		25	12	11.43^{defgh}	4.85^{jk}	83.72^{abcd}	45.17^{ghij}	38.55^{d}
		5	13	9.19^{ghi}	10.58^{cdef}	80.24^{bcde}	$48.23^{cdefghij}$	32.01^{ef}
5	150	15	14	12.71^{defgh}	8.10^{fghji}	77.79^{bcdef}	47.22^{efghij}	31.98^{ef}
		25	15	12.73^{defgh}	11.30^{cdef}	75.98^{cdef}	$49.09^{bcdefghij}$	26.90^{g}
		5	16	16.29^{cdefg}	12.84^{bcd}	70.88^{efghi}	51.65^{bcdefg}	22.16^{h}
	300	15	17	22.01^{bc}	11.45^{cdef}	66.64^{ghij}	51.80^{bcdef}	14.84^{j}
		25	18	18.61^{cd}	10.35^{cdef}	70.93^{efghi}	54.15^{abcd}	16.78^{ij}
		5	19	12.80^{defgh}	8.34^{fghi}	$78,87^{bcde}$	43.50^{ij}	35.37^{de}
	0	15	20	12.46^{defgh}	9.13^{defgh}	78.42^{bcde}	44.72^{ghij}	33.70^{ef}
		25	21	13.27^{defgh}	9.51^{cdefg}	77.22^{cdef}	42.19^{j}	32.98^{ef}
		5	22	14.66^{defgh}	10.52^{cdef}	74.82^{defg}	$51.28^{bcdefgh}$	31.29^{fg}
10	150	15	23	16.54^{cdefg}	10.27^{cdef}	72.19^{efghi}	51.58^{bcdefg}	20.62^{h}
		25	24	14.76^{defg}	5.89^{ghijk}	79.35^{bcde}	52.68^{abcde}	26.67^{g}
		5	25	15.62^{cdefg}	7.92^{fghij}	76.45^{cdef}	55.00^{abc}	21.46^{h}
	300	15	26	14.72^{defg}	8.56^{fghi}	76.43^{cdef}	55.25^{ab}	21.17^{h}
		25	27	18.30^{cde}	8.94^{efgh}	72.71^{efgh}	52.85^{abcde}	19.86^{hi}

*Treatment numbers **Average of 2 replications

a,b, Values with the same supperscript are not significantly (P>.05) different.

The figures higher than probably 10% may indicate some substances other than lignin itself since some lignin was already lost in the solution when sample was prepared. The most effective treatment was 0% NaOH under 150 PSI for 15 minutes (No. 5, 18.88%) which was different (P<.05) from the other treatment combinations except No. 6(15.90%). The figures of controls 1 (6.12%), 2 (3.97%) and 3 (4.16%) as well as other combinations which have less values than lignin content indicate that sodium chlorite did not completely remove lignin from these materials.

Pressure significantly (P<.01) changed *holocellulose content*, but neither NaOH nor time did. Holocellulose content was decreased (P<.05) from 84.79% to 74.81% and 69.18% as pressure level was changed from atmospheric pressure to 150 and 300 PSI, respectively. A two way interaction between NaOH and pressure was observed. At each level of NaOH, increase in pressure decreased holocellulose content. At atmospheric pressure it was consistently decreased as NaOH level increased, while at 300 PSI the content consistently increased. At 150 PSI it increased and then decreased as NaOH was changed from 0 to 5 and 10%, respectively.

Holocellulose content for controls was around 90%. Kamstra[4] have reported 78.4% for untreated bagasse and 44.2% for treated samples with 4% NaOH (w/w) at 400 PSI for 45 seconds. The lowest holocellulose content in the present study was 61.30% when treated with 0% NaOH under 300 PSI for 5 minutes (No. 7). These differences are probably due to (1) efficiency of alcohol extraction, (2) efficiency of delignification and (3) dry matter loss. Knap et al.[5] reported holocellulose content of 75.2% to 78.3% for untreated bagasse when benzen alcohol was used instead of alcohol alone.

Cellulose content was altered (P<.01) by pressure, but not by NaOH and time. Cellulose content was increased (P<.05) from 44.81% to 49.91% and 54.93% as pressure was increased from atmospheric to 150 and 300 PSI. However, an interaction between NaOH and pressure was observed. At atomospheric pressure, increase in NaOH level from 5% to 10% decreased cellulose content from 45.28% to 43.47%, while at 150 and 300 PSI the content was increased from 48.18% to 51.85% and from 52.53% to 54.36%, respectively. Although the relative cellulose content was increased by these treatment combinations compared to controls, the absolute content was decreased if dry matter loss was considered since differences in dry matter loss were larger than those in change in cellulose content. Crampton and Maynard[1] cellulose content showed a similar trend as reported herein. The values were higher than those of this separation method and the content ranged from 50.25% to 68.24%. Cellulose component of holocellulose was increased from 50% to 92%. This increase indicates a significant decrease in hemicellulose component of holocellulose. Kamstra[4] reported an increase in cellulose component of holecellulose from 46.5% to 67.2% when bagasse was treated with steam pressure alone at 400 PSI for 45 seconds where no dry matter loss was observed. By the same treatment the same worker found a decrease in Crampton and Maynard cellulose content from 44.9% to 37.5%. Ferguson[2] reported higher cellulose recovery in treated wheat straw than untreated one (43.78% vs 35.20%) with 20% dry matter loss due to washing. Ololade[6] found no significant differences in cellulose recovery between treated and untreated alfalfa stem, corn cobs and barley straw where no washing was followed and consequently no dry matter loss was observed.

Treatment with 0% NaOH under 300 PSI for 15 minutes (No. 8, 59.19%) showed the highest cellulose content, which was not significantly different from treatment 9 (59.16%), 7 (55.30%), 26 (55.25%), 25 (55.00%), 6 (54.29%), 18 (54.15%), 27 (52.85%) and 24 (52.68%), although different (P<.05) from the other treatment combinations including controls.

Hemicellulose (loss in KOH solution) was decreased (P<.01) by all three factors. The content was decreased (P<.05) from 39.92% to 25.91% and 14.55% as pressure was increased from atmospheric to 150 and 300 PSI, respectively. Zero percent NaOH (24.07%) was lower (P<.05) than 5% NaOH (29.31%), but not different from 10% NaOH (27.01%). Five minute treatment (30.17%) had higher (P<.05) hemicellulose content than 15 minutes (25.44%) and 25 miuntes (24.89%) although the table for these values is not presented here. A two way interaction between NaOH and pressure was found. The highest hemicellulose content was observed in controls and the lowest one in Nos. 7, 8 and 9. These results together with other criteria in cellulose preparation clearly suggest that more hemicellulose or soluble substances such as N.F.E. are lost, during the course of cellulose preparation, in the treatment combinations where lower loss in KOH solution was observed than in those where higher loss was found.

Correlations of sample recovery with criteria during cellulose preparation were examined and the results are shown in Table 4. Sample recovery was negatively correlated with alcohol extraction

Table 4. Correlations between sample recovery and products obtained during cellulose preparation

Correlation	Coefficient
Sample recovery × alcohol extraction	r = −0.7641**
Sample recovery × delignified lignin	r = −0.4181*
Sample recovery × holocellulose content	r = 0.7811**
Sample recovery × cellulose content	r = −0.9236**
Sample recovery × hemicellulose content	r = 0.8602**

*P<.05 **P<.01

(P<.01, r = -0.7641), delignification (P<.05, r = -0.4181) and cellulose content (P<.01, r = -0.9236), but positively correlated with holocellulose (P<.01, r = 0.7811) and hemicellulose (P<.01, r = 0.8602). Lower sample recovery or higher dry matter loss which indicates higher destruction of fiber system was related to increased alcohol extraction, delignification and apparent cellulose content and decreased holocellulose and hemicellulose contents.

IV SUMMARY

Hawaiian bagasse obtained from variety 50-7209, which consisted approximately 54% of sugarcane grown in the state, was used to study the effect of sodium hydroxide treatment of this material under steam pressure on the parameters such as alcohol extraction, delignification, holocellulose content and cellulose content involved in the process of bagasse cellulose preparation. Under the conditions employed in the present research the results are summarized as follows:

Pressure and time increased alcohol extractives. Delignification and relative cellulose content was also increased by pressure treatment though holocellulose content was decreased by the factor. All three factors had significant effect on hemicellulose content (loss in KOH solution). Two way interactions between pressure and NaOH were found in alcohol extractives, delignification, holocellulose content and cellulose content. Negative correlations of sample recovery were observed with alcohol extraction, delignification and cellulose content but positive with holocellulose and cellulose contents.

All these results clearly indicate that structure of bagasse material was chemically and physically altered by the treatment applied in this experiment. It was also found that the effect of pressure on the criteria studied was greater than NaOH and duration of the treatment.

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甘蔗バガスの加圧・NaOH処理による化学組成・消化率への影響

II. セルロース調製への影響

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要　　　約

ハワイで最も多く（54％）栽培されている品種，50－7209より得たバガスを材料として，圧力（大気圧，150 PSI，300 PSI），NaOH濃度（乾物に対し0％，5％，10％）および処理時間（5分，15分，25分）の三つの要因がバガスセルロース調製の過程に関与するアルコール抽出，脱リグニン，ホロセルロース，ヘミセルロースおよびセルロースなどの諸測定項目あるいはそれらの含量に対する効果を検討した。その結果を要約すると次の通りであった。

アルコール抽出物量は処理圧の増加，処理時間の延長により増加し，脱リグニン量およびセルロース含量は処理圧の増加により増量し，逆にホロセルロースの場合には減少した。ヘミセルロースは圧，NaOH濃度および処理時間の全要因の増加につれ減少した。圧とNaOH濃度間の相乗作用がアルコール抽出物量，リグニン量，ホロセルロース含量およびセルロース含量に認められた。さらに，処理後の原料回収量とアルコール抽出物量，脱リグニン量およびセルロース間に負の相関があった。逆に回収量とホロセルロースおよびセルロース量間においては正の相関が見られた。

以上の結果は甘蔗バガス繊維が圧力，NaOH濃度および処理時間の三要因により化学的あるいは物理的に変化したことを示唆するものであり，特に圧力要因の影響が大であった。

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