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# PROJECT REPORT FORM

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John W. Swanson

## FURTHER EXPERIMENTAL CONVERSIONS OF LOCUST BEAN GUM

### INTRODUCTION

This report deals with some experimental work on the conversion of locust bean gum by two additional methods, (1) degradation in hot glycerine and, (2) oxidation by acid dichromate solution.

### EXPERIMENTAL

#### I. Conversion Procedures

##### A. Glycerine Degradation of Gum G102-506

Forty grams of locust bean gum were mixed with 250 ml. of glycerine in a 400 ml. beaker and placed upon a small hot plate. The mixture was stirred mechanically while the temperature was raised to 170° C. during one hour. At this temperature bumping became so violent that it was necessary to turn off the heat supply and allow the mixture to cool to room temperature. The rather dark brown mixture was mixed with an equal volume of 95% ethyl alcohol and stirred vigorously for some time. The gum was filtered off on a Buchner funnel and resuspended in fresh alcohol, filtered and then extracted in a Soxhlet extractor for 13 hours with 85% methyl alcohol. The product was air dried for 2 days and possessed a light brown color.

##### B. Glycerine Degradation for Gum G107-506

Twenty-five grams of locust bean gum were mixed with 250 ml. of glycerine and heated on a hot plate as before. When the temperature reached 100° C., timing was started and heating was continued at such a rate that the temperature reached 217° C. in 23 minutes. The mixture was then allowed to cool rapidly to room temperature and was poured into 2 volumes of 95% ethyl alcohol, filtered and then extracted for 16 hrs. with 85% methyl alcohol. The gum was then air dried and appeared to be much darker brown in color than gum G102-506.

C. Potassium Dichromate Conversion G38-530 (1.6% available oxygen on gum).

Twenty grams of locust bean gum were dusted into 264 ml. of water in the steam cooker and then 30 ml. of 0.1 N potassium dichromate and 76 ml. of normal oxalic acid were stirred into the mixture. After about 10 minutes at room temperature, the yellow dichromate color disappeared and the viscous mixture became white. Steam was then injected at such a rate that the temperature was raised to 95° C. in ten minutes. During this time the color became a greenish violet and a rapid thinning out was noticed at about 85° C. Upon reaching 95° C. the mixture was weighed and immediately diluted to 2% gum concentration, cooled to 50° C. and used as a tub size on a 100% rag stock.

The above converted product as applied to the sheet was quite strongly acidic. Ten ml. of the gum solution were titrated with 0.1 N NaOH which indicated that the solution was 0.059 N in oxalic acid or that 17 ml. of the original oxalic acid had been consumed by the dichromate during the conversion period. In the next conversion a smaller relative amount of oxalic acid was used.

D. Potassium Dichromate Conversion G40-530 (4.8% available oxygen on the gum).

One hundred and twenty ml. of 0.1 N potassium dichromate solution and 30 ml. of N oxalic acid were mixed in the cooker and twenty grams of locust bean gum added with stirring. The mixture became quite viscous and the color gradually became violet. The reaction was allowed to proceed for one hour and twenty minutes during which time the temperature increased about 3° C. Then 280 ml. of water were stirred into the mixture and the temperature was raised to 95° C. in 15 minutes. Thinning of the solution was much slower during this conversion than with the G38-530 conversion. For this reason the temperature was held at 90° C. for 20 minutes more and then the mixture was diluted to 2% gum concentration and used as a tub size at 50° C. Titration of the gum solution showed that only 18 of the 30 ml. of oxalic acid were used up during the conversion.

E. Potassium Dichromate Conversion G44-530 (10% available oxygen on the gum).

Two hundred-fifty ml. of 0.1 N potassium dichromate, 76 ml. of N oxalic acid and 54 ml. of water were mixed together in the cooker and 20 g. of locust bean gum added with vigorous stirring. The mixture was allowed to react for one hour during which time 3° C. rise in temperature was noted. Then N sodium bicarbonate solution was added in 5 ml. portions until a pH of 6-7 was attained (required 40 ml.). After standing for one hour the temperature was raised to 95° C., held there for 5 minutes and then the solution was diluted to 2% gum concentration and used as a tub size at 50° C. Runs were made with both the usual high roll pressure and a low pressure. The latter was obtained by placing a fitted block of wood on the roll springs and adding two 1000 gram weights.

## II. Handsheet Evaluation of the Glycerine Converted Gums

The glycerine converted gums were evaluated both in the Waring Blendor and the Valley beater. The Waring Blendor evaluation was made in order to obtain some idea as to the dispersibility of the gums in comparison to the dispersibility of the alcohol acid hydrolyzed gums given in Report No. 1. The method of evaluation was the same and details may be found in Report No. 1 or pages 114-115 of notebook 506.

## III. Tub Size Evaluation of the Glycerine Converted Gums.

Eight grams of the gum (O.D. basis) were slurried in 524 ml. of water and the temperature was raised to 95° C. in 20 minutes. After being held at 90-95° C. for 5 minutes the mixture was diluted to one per cent gum concentration and used as a tub size in the small trough at 50° C.

## RESULTS AND DISCUSSION

### I. The Glycerine Converted Gums

It is quite evident from the data of Table I that the gums converted in glycerine were altered to a considerable extent. This is shown both by the relative viscosity measurements and the strength properties. The latter exhibit some interesting relationships. The burst, fold, etc. show that this type of conversion makes the locust bean gum more readily soluble in cold water. The fact that the G107-506 product gave lower values than G102-506

TABLE I

HANDSHEET CHARACTERISTICS OF THE GLYCERINE CONVERTED GEP'S WITH VARIOUS BLENDOR BEATER SULFITE  
 (GUNS ADDED DRY TO BEATER AND BEATER FOR FIVE MINUTES)

Gun Used	Relative Viscosity at 1% 30° C.	Basis Weight (500)	Bursting Strength (Mullen)		Per cent Increase in Burst	M/F Fold	Per cent Increase in Fold	Elmendorf Tear K./sheet	Tear Factor	Institute File No.	Condition of stained sheet
			Points	Pt. per 100 lb.							
Blank		47.2	10.3	22		4		103	2.18	110604	Very few specks
1 1/2 8102-506	25.0	47.3	17.5	37	63.2	14	250	122	2.56	110605	Many specks
1 1/2 8107-506	2.13	47.1	15.8	34	54.6	13	225	118	2.51	110606	Very few specks
1 1/2 Raw Gum	2100.0	47.3	15.3	32	28.0	9	125	110	2.33	110473	Many specks
Blank (for Raw Gum)		47.6	11.9	25	--	4	--	90	2.08		Very few specks
1 1/2 Raw Gum added cooked)		48.6	19.8	41	64.1	--	--	113	2.33	110866	A few specks

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TABLE IV

RELATION BETWEEN CUPRAMMONIUM FLUIDITY AND TENSILE STRENGTH OF VARIOUS HYDRO-CELLULOSES AND OXYCELLULOSES

Cuprammonium Fluidity Rhes	Loss in Tensile Strength From			
	Treatment with any acid %	Treatment with $K_2Cr_2O_7 + (COOH)_2$ %	Treatment with NaOCl %	Treatment with $K_2Cr_2O_7 + H_2SO_4$ %
10	10	1	7	2
20	34	6	25	11
30	58	16	47	26

some data on one of our better chlorinated products are included in Table V. The lower values obtained in the case of the product oxidized with sufficient dichromate to furnish 10% of oxygen on the gum may probably be accounted for either on the basis of over conversion or breakdown of the alkali-labile linkages due to the addition of the  $NaHCO_3$ . Further investigation of this point would be necessary for a definite conclusion.

On the basis of these results it would seem wise to investigate other types of concomitant oxidations which do not develop a poor color as a result of the reaction products. Several attacks along this line are being made at the present time with chlorine as the oxidizing agent.

SUMMARY

1. Several more converted locust bean products were made by two new methods (a) degradation in hot glycerine and (b) oxidation with acidic dichromate solution.

2. The glycerine converted products were found to be more readily dispersible in cold water than raw locust bean gum and one of the products was found to be almost the equivalent of cooked locust bean gum when evaluated in an unbeaten pulp.

3. The tub-size values of the glycerine products were not particularly outstanding. The bursting strength was fairly good but the folding endurance was poor.

4. The better dichromate converted products were evaluated as tub sizes and found to be distinctly superior to any converted gum thus far examined in this work.

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PROJECT REPORT FORM

PROJECT NO. 849 X  
COOPERATOR Institute  
REPORT NO. 13  
DATE November 24, 1943 - 12/1/43  
NOTE BOOK 416  
PAGE 62 TO 81  
SIGNED N. A. Kjelson

Copies to: Files - 849  
Mr. Steele  
Dr. Rowland

N. A. Kjelson

Subject:

Paper tests and other physical property measurements on various species of dry-milled endosperm mucilages.

General Procedures:

Most of the data given in this report had previously been transmitted to the interested parties and are compiled and analyzed here for the record. The data will be given in the order in which they were completed.

Three milled products were received from G.M.I. on October 1, 1943. These were as follows: (1) a methyl alcohol extracted guar mucilage G4-2, #R23624; (2) a new milling of flame tree mucilage G9, #R22640; (3) guar protein or embryo flour E4, #R23112 for tests of its properties in paper as well as tests on its effect upon other mucilages in paper.

The paper tests on these materials were carried out on the same pulp and by the procedures as described in the standard procedures in previous reports. Locust bean gum was again used as a control sample. Each was added in both the cooked and raw conditions with the exception of the E4 embryo which could not be dispersed in hot water and was therefore added in the uncooked condition only. The effect of the embryo material on a mucilage was first tried on locust bean gum where the cooked bean gum was added to the pulp suspension and then followed by the uncooked protein giving only a short period of contact time before the preparation of sheets. In a later series of experiments the protein material was first mixed with an endosperm mucilage suspension and then the mixture introduced into the pulp after a definite time. The sheets were again tested for formation, bursting strength and wet tensile, the latter test on selected sheets which were dipped into 1% borax solution and redried to set up the mucilage.

The test results are given in the accompanying tables I, II, and III.

TABLE I

THWING FORMATION NUMBERS OF SHEETS TREATED WITH THE COOKED AND UNCOOKED MUCILAGES

Mucilage and Method of Addition	2% Addition			1% Addition			1/2% Addition				
	Formation Number	% Inc. over Blank	Rank of Effectiveness	% of Result for Cooked Locust Bean Gum	Formation Number	% Inc. over Blank	Rank of Effectiveness	Formation Number	% Inc. over Blank	Rank of Effectiveness	
Guar G4-2 #R23624, uncooked	38.2	39.9	5	88.8	34.1	24.9	6	88.6	32.9	20.5	5
Guar G4-2 #R23624, cooked	37.0	35.5	6	86.0	36.1	32.2	4	93.8	30.9	13.2	6
Flame Tree G9 #R22640, uncooked	41.9	53.5	4	97.4	35.6	30.4	5	92.5	34.0	24.5	4
Flame Tree G9 #R22640, cooked	41.9	53.5	3	97.4	37.3	36.6	3	96.9	35.9	31.5	2
Locust Bean Gum, uncooked	43.0	57.5	2	--	38.5	41.0	2	--	35.5	30.0	3
Locust Bean Gum, cooked	48.0	75.8	1	112	42.2	54.6	1	110	41.2	50.9	1
Guar Embryo E4 #R23112, uncooked	32.7*				29.5	8.1	7	76.6	28.0	2.6	7

Blank Sheet  
 1% cooked locust bean gum plus 1/2% guar embryo protein E4 27.3 Formation Number  
 1% cooked locust bean gum plus 1% guar embryo protein E4 46.4 Formation Number  
 1% cooked locust bean gum plus 2% guar embryo protein E4 43.9 Formation Number  
 47.0 Formation Number

\* 8% Addition

TABLE II

BURSTING STRENGTHS (MULLEN) OF SHEETS TREATED WITH THE COOKED AND UNCOOKED MUCILLAGES

Mucilage and Method of Addition	2% Addition			1% Addition			1/2% Addition		
	Points	\$ Inc. over Blank	Rank of Effectiveness % of Result for Uncooked Locust Bean Gum.	Points	\$ Inc. over Blank	Rank of Effectiveness % of Result for Uncooked Locust Bean Gum.	Points	\$ Inc. over Blank	Rank of Effectiveness % of Result for Uncooked Locust Bean Gum.
Guar G4-2 #R23624, uncooked	28.4	59.6	5	27.0	51.7	3	23.4	31.5	4
Guar G4-2 #R23624, cooked	27.4	53.9	6	25.3	42.1	6	22.9	28.7	6
Flame Tree G9 #R22640, uncooked	31.0	74.2	3	26.4	48.3	4	23.2	30.3	5
Flame Tree G9 #R22640, cooked	32.3	81.5	1	28.1	57.9	2	26.0	46.1	2
Locust Bean Gum, uncooked	28.4	59.6	4	26.0	46.1	5	23.6	32.6	3
Locust Bean Gum, cooked	31.8	78.7	2	30.8	73.0	1	26.1	46.6	1
Guar Embryo E4 #R23112, uncooked	20.3*			17.8	0	7	68.5	--	7

Blank Sheet  
 1% cooked locust bean gum plus 1/2% guar embryo protein E4 17.8 Pt.  
 1% cooked locust bean gum plus 1% guar embryo protein E4 30.6 Pt.  
 1% cooked locust bean gum plus 2% guar embryo protein E4 29.6 Pt.  
 29.6 Pt.

\* 8% Addition

TABLE III

WET TENSILE STRENGTHS OF SHEETS TREATED WITH THE  
COOKED AND UNCOOKED MUCILAGES AND BORAX, IN UNITS PER 15 MM.

Mucilage and Method of Addition	2% Addition			1% Addition			1/2% Addition					
	Units	% Inc. over Blank	Rank of Effectiveness	% of Result for Uncooked Locust Bean Gum	Units	% Inc. over Blank	Rank of Effectiveness	% of Result for Uncooked Locust Bean Gum	Units	% Inc. over Blank	Rank of Effectiveness	% of Result for Uncooked Locust Bean Gum
Guar G4-2 #R23624, uncooked	6.4	357	6	83.1	5.5	293	5	91.7	4.2	200	4	93.3
Guar G4-2 #R23624, cooked	6.4	357	5	83.1	5.7	307	4	95.0	4.1	193	5	91.1
Flame Tree G9 #R22640, uncooked	7.2	414	4	93.5	5.2	271	6	86.7	3.8	171	6	84.4
Flame Tree G9 #R22640, cooked	7.6	443	3	98.7	6.2	343	2	103	4.9	250	2	109
Locust Bean Gum, uncooked	7.7	450	2	--	6.0	329	3	--	4.5	221	3	--
Locust Bean Gum, cooked	8.0	471	1	104	8.4	500	1	140	6.4	357	1	142
Guar Embryo E4 #R23112, uncooked	2.8*				1.7	21.4	7	28.3	1.4	0	7	31.1

Blank Sheet

1% cooked locust bean gum plus 1/2% guar embryo protein E4 1.4 Units  
 1% cooked locust bean gum plus 1% guar embryo protein E4 7.8 Units  
 1% cooked locust bean gum plus 2% guar embryo protein E4 7.3 Units  
 2% guar embryo protein E4 7.5 Units

\* 8% Addition

From these tables it can be seen that the samples tested are quite deficient in fiber deflocculating properties when compared with the cooked control sample, the locust bean gum. The flame tree bean mucilage, however, is better than the alcohol extracted guar sample and compares favorably when added cooked, with the results for the uncooked locust bean gum addition. The guar protein appears to have very little effect upon the formation although the small improvements noted are real. It has no deleterious effect upon the deflocculating properties of the control sample when added by the procedure described as indicated by the results at the bottom of Table I.

The cooked flame tree mucilage #R22640 shows itself to be almost as potent as an adhesive of the sort desired here as cooked locust bean gum, from Table II. The other samples and addition methods exhibit fairly good adhesive properties when compared with the uncooked controls but are definitely inferior to the control when added in its most effective form. The protein shows practically no adhesive properties and likewise has little effect upon the adhesive characteristics of a good mucilage incorporated with it.

Table III shows the wet tensile strength results for the sheets containing the various mucilages and given 1.0% sodium tetraborate dippings. All of the mucilage samples give fairly good results when compared with the blank (but dipped) sheet. However, the cooked control sample, the locust bean gum, is quite superior to the rest. The flame tree bean mucilage when added after cooking is about as effective as uncooked locust bean gum in this respect. The protein shows some slight positive results indicating that a small amount of manno-galactan is present as an impurity. No other material as far as is known will set up to a water-insoluble adhesive product with borax under the conditions described.

A report was received at this point which stated that the G9 flame tree bean mucilage #R22640 (the one tested above) showed little or no strength increases on a moderately highly hydrated furnish (M. & O. laboratory results). To disprove these results a furnish consisting of groundwood, unbleached sulfite, and bleached sulfate was beaten to the low freeness of 325 cc. Sch.-R. at 20° C. Rosin size and alum were added by the conventional method toward the end of the beating cycle. Additions of the mucilages at the dilute concentrations of 1/2 and 1% were made to the beaten pulp as described in Table IV. These materials were added to the pulp on the basis of 11% moisture content and the percentage figures given are based on these weights and the oven-dry content of the pulp furnish

TABLE IV

SHEET PROPERTIES OF A GROUNDWOOD-SULFITE-SULFATE FURNISH  
WITHOUT AND WITH THE MUCILAGES AND EMBRYO PROTEIN

Mucilage and Method of Addition	Thwing Formation		Bursting Strength		Folding Endurance		Tensile Strength	
	Number	% Increase over the Blank	Points Mullen	% Increase over the Blank	M.I.T. Folds	% Increase over the Blank	Pounds per Inch Strip	% Increase over the Blank
1/2% cooked G9 #R22640	43.9	8.13	27.8	7.75	89	20.3	18.9	5.59
1% cooked G9 #R22640	45.6	12.3	28.2	9.30	101	36.5	19.5	8.94
1/2% cooked G4 #R22378	40.7	.25	27.0	4.65	155	109.	19.3	7.82
1% cooked G4 #R22378	43.2	6.40	27.5	6.59	101	36.5	19.9	11.2
1/2% cooked G4 plus 1/4% E4	44.4	9.36	26.2	1.55	124	67.6	19.1	6.70
1% cooked G4 plus 1/2% E4	44.7	10.1	27.6	6.98	135	82.4	19.9	11.2
Untreated Sheet	40.6		25.8		74		17.9	

†

The powdered mucilages were weighed on the analytical balance, their moisture contents having been previously determined, and dusted into stirring 80° C. water on a water bath. The G9 mucilage was cooked at 1/2% solids for 10.0 minutes at 80° C. and cooled by top water before addition to the beater pulp suspension. The yellow guar mucilage # R22378 was cooked at 1.0% solids and after cooling, was homogenized in the Waring Blendor at 1.0% solids, the Blendor running at top speed for 3.0 minutes. This was followed by dilution to 1/2% solids using the dilution water for rinsing the homogenizer. This gave a very viscous suspension at 1/2% solids. It was free of the large undispersed particles which were present after the cooking procedure. After this mucilage suspension was applied in the first series of experiments, it was mixed with 1/2 volume of 1/2% solids cooked guar embryo E4 #R23112. This was done to determine whether the presence of the protein had any effect upon the desired properties of the mucilage. The E4 protein was first cooked alone at 80° C. for 10 minutes and cooled. The data in Table IV show the following: definite and marked improvements in the formation, bursting strength, folding endurance and tensile strength are exhibited by these small amounts of gum on this low-freeness, high-groundwood-content furnish. For instance, the 1% concentration of the flame tree bean mucilage gives a 12% increase in formation number, a 9.3% increase in bursting strength, a 36.5% increase in fold and an 8.9% increase in tensile. There is no reason to believe that these improvements could not be achieved in mill operation provided the stuff were properly handled. The presence of the protein does not interfere with the strength giving properties of the guar mucilage as indicated, and aids its fiber deflocculating properties since better formation results are obtained where it is used with the mucilage.

Viscosity tests were made on this new G9 mucilage. The same pipette and conditions were employed as for the viscosity tests in previous reports. In addition, a portion of the powdered G9 was cooked in water made alkaline to pH 9 to 9.5 with NaOH for viscosity tests with and without toluene as a preservative both before and after aging for various periods of time. For comparison with the previous G9 sample, a dispersion in distilled water was first prepared by cooking according to the standard schedule. The viscosity results, which should be compared with those obtained previously, are given in Table V. The solids figures given are based on a mucilage containing 11% of moisture.

Table V

Pipette Viscosity of G9 #R22640 Mucilage at 30° C.

<u>Percent Solids</u>	<u>Viscosity Seconds</u>	<u>Relative Viscosity</u>
0.5%	18.9	3.600
0.4%	14.2	2.705
0.3%	10.9	2.076
0.2%	8.5	1.619
0.1%	6.7	1.276

For the alkali cook of sample G9 #R22640, two liters of tap water were treated with 2.0 cc. of 5.0% NaOH solution. This produced a pH between 9.0 and 9.5. Eight hundred cc. of this water were heated to 30° C. and the weighed amount of G9 powder dusted in and cooked as usual. No differences were noted in the appearance of the alkali cooked G9 when compared with the nonalkali cooked batch above. Ostwald pipette viscosity tests on the 0.50% solids sample gave 19.0 seconds or a result practically identical with the sample cooked above. A volume of about 70 cc. of this alkaline sample and an equal volume sample but containing an added 1.0 cc. of toluene were stored in separate stoppered test tubes in a constant temperature water bath at 30° C. for viscosity tests after aging. In addition the guar mucilage G<sup>h</sup>-2 #R22602 after suspending in cold water was also aged at 30° C. in stoppered tubes but containing (a) no preservative, (b) formalin, (c) toluene. A weighed amount of the R22602 was dusted into room temperature distilled water and stirred for a period of 1/2 hour. At the end of this period, the contents were weighed and found to be 3.0 g. light. The suspension was then divided into three parts and 1 g. of distilled water added to the first, 1 g. of formalin to the second, and 1g. of toluene to the third. The viscosities were then run at 30.0° C. and the samples stored for aging as above. The aging at 30° C. was carried out for as long as 208 hours. These aging experiments were carried out to attempt to explain the reasons for the degradation and reduction in viscosity on standing in non-sterile and sterile conditions. It was thought that if the degradation were due to contamination by bacteria from the air, that both toluene and formalin would be able to stop the action, and that if it were due to a natural self-contained enzyme of a protein nature, that formaldehyde might inactivate it while toluene might not. The data are not complete enough to form any definite conclusions since only one aging temperature was used and no bacteriological tests were made on the so-called sterilized samples to determine the exact state of their sterility--for instance, enough of the toluene might have evaporated during the period of removal of sample, that its effect as a bactericide might have been negated toward the end of the experiment. The results are given in Table VI.

TABLE VI

Ostwald Pipette Viscosities\* at 30.0° C. of 0.50% Solids Mucilage Suspensions Aged for Various Periods at 30° C. in a Water Bath, in Seconds

Mucilage and Preservative	Immediately						
	after Frep.	Aged 16 hr.	Aged 40 hr.	Aged 64 hr.	Aged 88 hr.	Aged 112 hr.	Aged 208 hr.
G9 cooked at pH 9, No Pres.	19.0 sec.	16.9	9.5	0.2		5.7	Discarded
G9 cooked at pH 9, toluene added		17.2	13.8	0.7		5.9	Discarded
G4-2 Uncooked, No Pres.	24.5	14.8	14.7		12.5		Futrified
G4-2 Uncooked, with toluene	23.9	15.4	12.5		10.1		8.4
G4-2 Uncooked, with formalin	24.1	17.3	15.7		14.2		11.8

\* Water at this temperature runs 5.25 seconds in this pipette

Two samples of guar G4-2 mucilage were received from G.M.I. for paper tests and viscosity measurements. These two samples, #R23830 and #R23831, were alcohol precipitated fractions of a protein-free and seed coat-free G4-2 milled guar prepared by hot water suspending, purification by centrifuging and fractionally precipitating with methyl alcohol. The R23830 was a fibrous type of mucilage while the other was a non-fibrous or granular type of mucilage when dry. Both of these samples as well as flame bean mucilage and locust bean gum were cooked by the standard procedure before the usual paper tests on lightly beaten bleached sulfite. Numerous dough-balls were present in the cooked alcohol-precipitated mucilages. In order to smooth these out, each was given two passes through the hand operated homogenizer (mild action) after cooling to room temperature. The Ostwald pipette viscosity ( $H_2O = 5.25$  sec. at 30° C.) of these 0.50% suspensions were: #R23830 - 73.6 seconds and for the R23831 - 146.0 seconds. The resultant sheet properties are displayed in Table VII. It can be readily seen from the table that although the non-fibrous guar fraction was more viscous than the fibrous fraction, the fiber dispersing powers are no greater as indicated from the formation results. The flame bean mucilage which has quite a low viscosity is, suprisingly enough, a better fiber deflocculant



than the more viscous and purer guar samples. It does not, however, approach that of the very much more viscous locust bean gum. An explanation for these phenomena would be a conjecture at the present time, but it is evident that the three factors of (1) length of the molecule, (2) shape of the molecule and (3) degree of degradation from their natural state, are involved. The bursting strength results again show that this sample of flame bean mucilage is about equal to locust bean gum as an adhesive. Both of these outrank the guar samples, and of the latter, the fibrous type is definitely more potent. Excellent water-proof adhesives are obtained from all of the samples from a study of the wet tensile strength data, but the locust bean gum still leads the list. The borax gel from the fibrous guar is stronger than that from the non-fibrous. The Ostwald pipette viscosities of the cooked 0.50% milled samples were: flame bean No. R22640 - 19.3 seconds; locust - 428.0 seconds at 30° C.

#### Tests on Commercial Papers:

Mill laboratory trials at Minnesota and Ontario Paper Company with the G9 flame bean mucilage No. R22640 indicated that although they obtained a 60% increase in bursting strength of their kraft sheet with the cooked material when added to a lightly beaten furnish, little benefit was derived from it at papermaking freenesses. For trials in this laboratory, large samples of M. and O. wet lap kraft and unbleached sulfite pulps were obtained. For the kraft pulp a standard beater experiment was made, with the beating intervals: 10, 40, 70 and 100 minutes. Two 10 g. (O.D.) batches of pulp were removed at each interval, one for the untreated systems and one for the 2.0% additions of cooked G9. The test results are given in Table VIII. The data do not form as smooth curves as expected, but they certainly disprove the statement that little effect will be produced by this gum on highly hydrated kraft. Note especially the 100 minute beating interval in Table VIII. Here the pulp is beaten far beyond that ever used commercially for this pulp, to a Schopper-Riegler freeness of 190 cc., but still there is the remarkable increase in bursting strength by the gum of 22.6% over the untreated sheet. There is also a 20% increase in folding endurance and over a 10% increase in tensile strength. A freeness of 465 cc. as in the 70 minute beating interval is probably closer to what the pulp would usually run on a machine (or possibly slightly less beaten) and if a mill could consistently obtain the improvements shown for this 70 minute interval by 2% of this mucilage, i.e. 11.3% increased bursting strength, 22.1% increased folding endurance and 7.14% increased tensile strength, it would pay a good price for the material even though it were used on as cheap a sheet as a kraft.

TABLE VIII

SHEET TEST RESULTS OF M. AND O. KRAFT  
WITHOUT AND WITH G9 MUCILAGE

	Without Additive	With 2% Cooked G9 No. R22640
<b>10 Minute Beating Interval</b>		
Freeness, Sch-R. 20° C., cc.	835	830
Sheet Basis Weight, 25x40/500, lb.	54.8	53.0
Bursting Strength, pt./100 lb.	104	133
Burst, % Inc. over Untreated Sheet		27.9
Folding Endurance, M.I.T.	1134	907
Fold, % Inc. over Untreated Sheet		--
Tensile Strength, lb./in.	27.2	31.0
Tensile, % Inc. over Untreated Sheet		14.0
<b>40 Minute Beating Interval</b>		
Freeness, Sch-R. 20° C., cc.	760	735
Sheet Basis Weight, 25x40/500, lb.	53.6	52.7
Bursting Strength, pt./100 lb.	143	173
Burst, % Inc. over Untreated Sheet		21.0
Folding Endurance, M.I.T.	1273	1361
Fold, % Inc. over Untreated Sheet		6.91
Tensile Strength, lb./in.	38.1	40.2
Tensile, % Inc. over Untreated Sheet		5.51
<b>70 Minute Beating Interval</b>		
Freeness, Sch-R. 20° C., cc.	465	420
Sheet Basis Weight, 25x40/500, lb.	51.7	51.8
Bursting Strength, pt./100 lb.	151	168
Burst, % Inc. over Untreated Sheet		11.3
Folding Endurance, M.I.T.	1324	1616
Fold, % Inc. over Untreated Sheet		22.1
Tensile Strength, lb./in.	40.6	43.5
Tensile, % Inc. over Untreated Sheet		7.14
<b>100 Minute Beating Interval</b>		
Freeness, Sch-R. 20° C., cc.	190	165
Sheet Basis Weight 25x40/500, lb.	52.8	51.3
Bursting Strength, pt./100 lb.	137	168
Burst, % Inc. over Untreated Sheet		22.6
Folding Endurance, M.I.T.	1432	1721
Fold, % Inc. over Untreated Sheet		20.2
Tensile Strength, lb./in.	37.8	41.8
Tensile, % Inc. over Untreated Sheet		10.6

That M. and O. unbleached sulfite is more responsive to the action of the G9 mucilage than is their kraft, is shown in Table IX. Only two beating intervals are included here because the pulp was of the rapid beating type (possibly high pentosan content) and the 70 minute beating interval from which no sheets were prepared, had a freeness of 100 cc.--so low that the sheets could not be couched properly. But note the excellent strength increases at the 10 minute beating interval: 32.1% increased bursting strength, 194.4% increased folding endurance and 29.9% increased tensile strength. It is also interesting to note that the sheet beaten for 10 minutes and containing the mucilage is much stronger than the more highly beaten sheet of the 40 minute interval containing no additive and which is probably quite close to its natural maximum strength. This result might indicate that the adhesive properties of this mucilage when applied by the method used, is stronger than the gelatinous material obtained from this pulp on beating.

TABLE IX

TEST RESULTS OF G9 MUCILAGE ON M. AND O. UNBLEACHED SULFITE PULP

	Without Additive	With 2% Cooked G9 Mucilage No. R22640
<b>10 Minute Beating Interval</b>		
Freeness Sch.-R. 20° C., cc.	745	675
Sheet Basis Wt. 25x40/500, lb.	51.2	50.9
Bursting Strength pts./100 lb.	69.7	92.1
Burst, % Inc. over Untreated Sheet		32.1
Folding Endurance M.I.T.	107	315
Fold, % Inc. over Untreated Sheet		194.4
Tensile Strength, lb./in.	21.1	27.4
Tensile, % Inc. over Untreated Sheet		29.9
<b>40 Minute Beating Interval</b>		
Freeness Sch.-R. 20° C., cc.	345	225
Sheet Basis Wt. 25x40/500, lb.	51.0	50.9
Bursting Strength pts./100 lb.	76.7	91.6
Burst, % Inc. over Untreated Sheet		19.4
Folding Endurance	272	457
Fold, % Inc. over Untreated Sheet		68.0
Tensile Strength, lb./in.	25.9	30.0
Tensile, % Inc. over Untreated Sheet		15.8

"King William" butcher wrap pulp was received from Nekoosa Paper Company for tests with G9 No. R22640 as an additive. This was done as a preliminary to the mill trial tests on this material. The pulp was shipped from the mill after going through its regular beating and jordaning for the manufacture of the commercial sheet. The furnish for this grade is a mixture of unbleached sulfite and semi-bleached kraft (40-60?) containing size and alum and given only a moderate beating. The Schopper-Riegler freeness of the sample received was 720 cc. at 20° C. The G9 mucilage was cooked and applied to the pulp at the concentrations of 0, 1/2, 1, and 2% solids based on the oven dry weight of the pulp. The freeness of the stock containing 1/2% of mucilage was 695 cc. and that containing 2%, 665 cc. Bursting strength, fold and dry tensile were run on the sheets, the results of which are shown in Table X. The strength improvements due to the G9 are quite large. There is no reason to believe that these results cannot be equaled or exceeded in mill operation. In fact a less brittle sheet for better wrapping should be made by less beating. It should then show greater all around strength properties including tear.

TABLE X

KING WILLIAM BUTCHER WRAP PULP,  
 BEATEN AND JORDANED AT NEKOOSA,  
 WITHOUT AND WITH COOKED G9 AS ADDITIVE

	Bursting Strength pt/100 lb.	Burst % Inc. over blank	M.I.T. Fold	Fold % Inc. over blank	Tensile lb/in	Tensile % Inc. over Blank	Basis Wt. 25/40 500, lb.
Pulp as received no additive	83.9		304		25.6		55.0
Pulp plus 1/2% cooked G9	89.6	6.79	519	70.7	27.3	6.64	55.6
Pulp plus 1% cooked G9	93.1	11.0	524	72.4	27.8	8.59	56.7
Pulp plus 2% cooked G9	95.6	14.0	519	70.7	28.1	9.77	55.1

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COOPERATOR Institute  
REPORT NO. 5  
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PAGE 38-45 TO 70  
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John W. Swanson

## FURTHER EXPERIMENTAL CONVERSIONS OF LOCUST BEAN GUM

### INTRODUCTION

This report deals with some experimental work on the conversion of locust bean gum by two additional methods, (1) degradation in hot glycerine and, (2) oxidation by acid dichromate solution.

### EXPERIMENTAL

#### I. Conversion Procedures

##### A. Glycerine Degradation of Gum G102-506

Forty grams of locust bean gum were mixed with 250 ml. of glycerine in a 400 ml. beaker and placed upon a small hot plate. The mixture was stirred mechanically while the temperature was raised to 170° C. during one hour. At this temperature bumping became so violent that it was necessary to turn off the heat supply and allow the mixture to cool to room temperature. The rather dark brown mixture was mixed with an equal volume of 95% ethyl alcohol and stirred vigorously for some time. The gum was filtered off on a Buchner funnel and resuspended in fresh alcohol, filtered and then extracted in a Soxhlet extractor for 13 hours with 85% methyl alcohol. The product was air dried for 2 days and possessed a light brown color.

##### B. Glycerine Degradation for Gum G107-506

Twenty-five grams of locust bean gum were mixed with 250 ml. of glycerine and heated on a hot plate as before. When the temperature reached 100° C., timing was started and heating was continued at such a rate that the temperature reached 217° C. in 23 minutes. The mixture was then allowed to cool rapidly to room temperature and was poured into 2 volumes of 95% ethyl alcohol, filtered and then extracted for 16 hrs. with 85% methyl alcohol. The gum was then air dried and appeared to be much darker brown in color than gum G102-506.

C. Potassium Dichromate Conversion G38-530 (1.6% available oxygen on gum).

Twenty grams of locust bean gum were dusted into 264 ml. of water in the steam cooker and then 80 ml. of 0.1 N potassium dichromate and 76 ml. of normal oxalic acid were stirred into the mixture. After about 10 minutes at room temperature, the yellow dichromate color disappeared and the viscous mixture became white. Steam was then injected at such a rate that the temperature was raised to 95° C. in ten minutes. During this time the color became a greenish violet and a rapid thinning out was noticed at about 85° C. Upon reaching 95° C. the mixture was weighed and immediately diluted to 2% gum concentration, cooled to 50° C. and used as a tub size on a 100% rag stock.

The above converted product as applied to the sheet was quite strongly acidic. Ten ml. of the gum solution were titrated with 0.1 N NaOH which indicated that the solution was 0.059 N in oxalic acid or that 17 ml. of the original oxalic acid had been consumed by the dichromate during the conversion period. In the next conversion a smaller relative amount of oxalic acid was used.

D. Potassium Dichromate Conversion G40-530 (4.8% available oxygen on the gum).

One hundred and twenty ml. of 0.1 N potassium dichromate solution and 30 ml. of N oxalic acid were mixed in the cooker and twenty grams of locust bean gum added with stirring. The mixture became quite viscous and the color gradually became violet. The reaction was allowed to proceed for one hour and twenty minutes during which time the temperature increased about 3° C. Then 280 ml. of water were stirred into the mixture and the temperature was raised to 95° C. in 15 minutes. Thinning of the solution was much slower during this conversion than with the G38-530 conversion. For this reason the temperature was held at 90° C. for 20 minutes more and then the mixture was diluted to 2% gum concentration and used as a tub size at 50° C. Titration of the gum solution showed that only 18 of the 30 ml. of oxalic acid were used up during the conversion.

E. Potassium Dichromate Conversion G44-530 (10% available oxygen on the gum).

Two hundred-fifty ml. of 0.1 N potassium dichromate, 76 ml. of N oxalic acid and 54 ml. of water were mixed together in the cooker and 20 g. of locust bean gum added with vigorous stirring. The mixture was allowed to react for one hour during which time 8° C. rise in temperature was noted. Then N sodium bicarbonate solution was added in 5 ml. portions until a pH of 6-7 was attained (required 40 ml.). After standing for one hour the temperature was raised to 95° C., held there for 5 minutes and then the solution was diluted to 2% gum concentration and used as a tub size at 50° C. Runs were made with both the usual high roll pressure and a low pressure. The latter was obtained by placing a fitted block of wood on the roll springs and adding two 1000 gram weights.

## II. Handsheet Evaluation of the Glycerine Converted Gums

The glycerine converted gums were evaluated both in the Waring Blendor and the Valley beater. The Waring Blendor evaluation was made in order to obtain some idea as to the dispersibility of the gums in comparison to the dispersibility of the alcohol acid hydrolyzed gums given in Report No. 1. The method of evaluation was the same and details may be found in Report No. 1 or pages 114-115 of notebook 506.

## III. Tub Size Evaluation of the Glycerine Converted Gums.

Eight grams of the gum (O.D. basis) were slurried in 524 ml. of water and the temperature was raised to 95° C. in 20 minutes. After being held at 90-95° C. for 5 minutes the mixture was diluted to one per cent gum concentration and used as a tub size in the small trough at 50° C.

## RESULTS AND DISCUSSION

### I. The Glycerine Converted Gums

It is quite evident from the data of Table I that the gums converted in glycerine were altered to a considerable extent. This is shown both by the relative viscosity measurements and the strength properties. The latter exhibit some interesting relationships. The burst, fold, etc. show that this type of conversion makes the locust bean gum more readily soluble in cold water. The fact that the G107-506 product gave lower values than G102-506

TABLE I

HANDBEAT CHARACTERISTICS OF THE GLYCERINE CONVERTED GUMS WITH HAVING BLENDOR BEATEN SULFITE  
 (GUMS ADDED DRY TO BEATER AND BEATEN FOR FIVE MINUTES)

Gum Used	Relative Viscosity at 1% .30° C.	Basic Weight 25x40/500	Bursting Strength (Mullen) Pt. per 100 lb.	Per cent Increase in Burst	Mif Fold	Per cent Increase in Fold	Blmendorf Tear g./sheet	Tear Factor	Institute File No.	Condition of stained sheet
Blank		47.2	22		4		103	2.18	110604	Very few specks
1% G102-506	25.0	47.3	37	65.2	14	250	122	2.53	110605	Many specks
1% G107-506	2.43	47.1	34	54.6	13	225	114	2.51	110606	Very few specks
1% Raw Gum	21000.0	47.3	32	28.0	9	125	110	2.33	110423	Many specks
Blank (for Raw Gum)		47.6	25	--	4	--	99	2.08		Very few specks
1% Raw Gum added cooked		48.6	41	64.1	-	--	113	2.33	110866	A few specks

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indicates that molecular degradation in the former had taken place to an excessive degree. The reason for the low strength features exhibited by raw locust bean gum is shown by the condition of the stained sheet which proved that this gum did not disperse very well in the cold water used for beating. The most significant point about this table is that the G102-506 product was almost equivalent in bursting strength to cooked raw locust bean gum despite the fact that it was not completely dispersed as shown by the staining technique. The strength features of this gum obtained by adding the dry gum to the Valley beater during the beating procedure seem to be fairly good also. (See Table II).

The tub size characteristics of these gums are given in Table III. The sizings were made at 1% gum concentration because of the rather high viscosity of the G102-506 product. The bursting strength values are fairly good for a one per cent tub size but the folding endurance was found to be quite low.

## II. The Dichromate Converted Gums.

An explanation of this conversion should be made prior to discussion of the data obtained.

It was realized prior to the beginning of these conversions that the poor color developed would prohibit practical application of the method. The principal purpose was to test the applicability of certain data in the literature to conversion of mannogalactan gums. Clibbens and Ridge (J. Textile Inst. 19T389(1928)), during an investigation of the degradative effects of various reagents on cellulose, found that any acid gave the same cuprammonium fluidity and decrease in tensile strength, but that a similar relationship did not exist for all types of oxycellulose. This is brought out by some of their data which is reproduced below in Table IV. According to this data, the use of a mixture of potassium dichromate and oxalic acid should convert the gum and retain a high percentage of the original adhesive strength providing the material is maintained in an acidic or neutral condition.

The tub-size data are presented in Table IV, and it is believed that this does bear out the idea presented. The burst and fold values obtained by tub-sizing with the converted dichromate products are distinctly superior to any received so far by other types of conversion. For comparison

TABLE IV

## RELATION BETWEEN CUPRAMMONIUM FLUIDITY AND TENSILE STRENGTH OF VARIOUS HYDRO-CELLULOSES AND OXYCELLULOSES

Cuprammonium Fluidity Rhes	Loss in Tensile Strength From			
	Treatment with any acid %	Treatment with $K_2Cr_2O_7 + (COOH)_2$ %	Treatment with NaOCl %	Treatment with $K_2Cr_2O_7 + H_2SO_4$ %
10	10	1	7	2
20	34	6	25	11
30	58	16	47	26

some data on one of our better chlorinated products are included in Table V. The lower values obtained in the case of the product oxidized with sufficient dichromate to furnish 10% of oxygen on the gum may probably be accounted for either on the basis of over conversion or breakdown of the alkali-labile linkages due to the addition of the  $NaHCO_3$ . Further investigation of this point would be necessary for a definite conclusion.

On the basis of these results it would seem wise to investigate other types of concomitant oxidations which do not develop a poor color as a result of the reaction products. Several attacks along this line are being made at the present time with chlorine as the oxidizing agent.

## SUMMARY

1. Several more converted locust bean products were made by two new methods (a) degradation in hot glycerine and (b) oxidation with acidic dichromate solution.

2. The glycerine converted products were found to be more readily dispersible in cold water than raw locust bean gum and one of the products was found to be almost the equivalent of cooked locust bean gum when evaluated in an unbeaten pulp.

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3. The tub-size values of the glycerine products were not particularly outstanding. The bursting strength was fairly good but the folding endurance was poor.

4. The better dichromate converted products were evaluated as tub. sizes and found to be distinctly superior to any converted gum thus far examined in this work.

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## SOME EXPERIMENTS WITH A POSSIBLE FRACTIONATION OF LOCUST BEAN GUM AND EVALUATION OF THE PRODUCTS

### Introduction

During the latter part of May, 1943, workers of General Mills, Inc. stated that they had obtained an alleged fractionation of Honey locust bean mucilage. Two principal fractions were obtained by extracting the crude gum with cold water. These were designated as cold water soluble and cold water insoluble fractions and according to their statements possessed the following viscosities.

TABLE I

MACMICHAEL VISCOSITIES OF GMI FRACTIONS AT 25° C. AND 0.5% CONCENTRATION

Fraction	Previous Treatment	Viscosity ° m. No. 130 Wire
C.W.S.	Dissolved in cold water	140
C.W.S.	Dissolved in cold water and heated to 95° C. and cooled to 25° C.	199
C.W.I.	Dissolved in hot water, cooled to 25° C.	317

The viscosity of the cold water insoluble fraction was stated to be greater than that of the whole mucilage.

We became interested in the possibility of fractionation of the mucilages because of the relationship to our work on converted locust bean gum. If two distinct fractions, possessing different chemical or physical

properties, are present in the gums, converting agents might possibly attack one fraction in preference to the other under certain experimental conditions. Therefore, it seemed worth while to investigate the properties of the fractions and to study their behavior under conversion procedures previously used on the whole gum (see Report No. 1). This report presents the work done on preliminary fractionation and an investigation of the effects of acid hydrolysis on two arbitrarily chosen fractions. An extensive investigation of the fractionation and colloidal properties of the fractions has been undertaken as a Ph.D. thesis problem.

#### Work Done

1. Locust bean gum was exhaustively extracted with cold water followed by hot water extraction of the residue. This was repeated with a Guar mucilage.
2. An arbitrary fractionation was decided upon and the properties of the products such as relative viscosity, optical rotation, ash, phosphorous, calcium, and nitrogen were determined.
3. The arbitrary fractions and their hydrolysed counterparts were evaluated in handsheets.

#### Experimental

Extraction of Locust Bean Gum with Cold Water. Fifteen grams of air dried locust bean gum were stirred with three liters of distilled

water for two hours at room temperature (26-27° C.). The mixture was then supercentrifuged and the liquid extract poured into an equal volume of acetone which precipitated the dissolved gum as a white, stringy mass. This was dehydrated in fresh acetone, air dried, and labeled as fraction No. 1--Yield 6.6 grams. The undissolved residue of 68.9 grams of wet gel was suspended in 500 ml. of water and stirred for 16 hours. This mixture was centrifuged and the extract precipitated with acetone. The residue was mixed with 225 ml. of water in a centrifuge bottle and stirred for two hours, centrifuged and the extract precipitated. This procedure was then repeated a total of 10 extractions. The tenth extraction was continued for seven hours and yielded such a small quantity of extracted gum that the cold water treatment was substituted by a one hour extraction at 92° C. This procedure yielded only a very small amount of extracted material. The hot water insoluble residue weighed 1.1 grams which is 3.2% of the original gum.

A similar series of extractions were made on Guar mucilage, the first extract yielding 8.3 grams. The eighth extract of this mucilage gave only an opalescence when poured into acetone so the one hour hot water extract was made. This extract yielded only a very small quantity of extracted material. The hot water insoluble residue weighed 1.3 grams which is 9.6% of the original gum.

The Arbitrary Fractionation Procedure. Most of the work was done on just two fractions, a cold water extracted product and a hot water soluble product extracted from the residue.

Fifteen grams of the raw locust bean gum were stirred with three liters of cold water (about 25-27° C.) for two hours, and the mixture was supercentrifuged. The undissolved residue was extracted with 1500 ml. of hot water (92° C.) for one hour and then supercentrifuged. The extracts were poured into equal volumes of acetone, and the precipitated products were dehydrated with fresh acetone and air dried. These products were designated as the cold water soluble fraction (C.W.S.) and the hot water soluble fraction (H.W.S.). The quantities of air dried fractions obtained were as follows:

C.W.S.	6.7 grams	44.7%
H.W.S.	4.2 grams	28.0%
Residue	1.1 grams	7.3%
Total	12.0 grams	80.0%

Part of the twenty per cent loss of original gum may be accounted for as fat solvent soluble material. An 85% methanol extraction of the raw gum yielded 3.9% of oily material.

The following analyses of the fractions were made.

TABLE II  
ANALYSES OF THE ARBITRARY FRACTIONS OF LOCUST BEAN GUM

	C.W.S. Fraction	H.W.S. Fraction
Moisture %	12.22	11.95
Ash* %	0.23	0.09
Phosphorus* %	0.014	0.010
Calcium* %	0.049	0.038
Nitrogen* %	0.00	0.00

\* Oven-dry basis

Fractionation of the 20-Minute Hydrolyzed Gum G103-431. This gum is the same as that used in Report No. 1, and its fractionation was carried out similarly to that of the arbitrary fractionation of the whole gum. The yield of each fraction from 15 grams of the gum follows below.

H - C.W.S.	9.53 grams	63.6%
H - H.W.S.	1.68 grams	11.2%
Residue	.58 grams	3.9%
Total	11.79 grams	78.7%

Hydrolysis of the Fractions of Locust Bean Gum. In order to interpret and compare the properties of the fractions more closely with those of the hydrolyzed whole gums the fractions were hydrolyzed similarly to the procedure used for the whole gum.

Five grams of the fraction which had been ground to pass a 50 mesh sieve were mixed with 25 ml. of acidic ethyl alcohol. The acid alcohol was made by mixing 3 ml. of concentrated HCl with 400 ml. of 95% ethyl alcohol. The flask was then connected to a reflux condenser and heated on a steam bath for an exact interval--either five or ten minutes as desired. Then 2.5 ml. of normal  $\text{NaHCO}_3$  solution were added with shaking to neutralize the acid. The mixture was then filtered on a Büchner funnel and the gum fraction washed several times with fresh 95% alcohol, air dried and then vacuum dried over  $\text{P}_2\text{O}_5$  at room temperature.

Determination of the Relative Viscosities of the Fractions. The relative viscosities were determined on 0.3% dispersions at 30° C. as follows.

A 0.2 gram sample of the air dried fraction of known moisture content was weighed out in a 100 ml. beaker along with a small glass stirring rod. The mixture was wetted with a little water and then diluted to 50 ml. In some cases the C.W.S. fraction was dispersed by continuous stirring at room temperature, but this required 16-30 hours of continuous stirring. More rapid dispersion was accomplished by suspending the beaker about 1/4 inch above a small hot plate which raised the temperature to about 70° C. This procedure was necessary with the H.W.S. fraction and required about five hours for visual clarity. For complete hydration of this fraction at least a momentary temperature of 92° C. was necessary for valid viscosity

measurements. When dispersion appeared to be complete the mixture was cooled to room temperature and diluted to the correct weight for a 0.3% gum dispersion. Ten ml. samples were then pipetted into Ostwald viscometers and the viscosity determined in the regular manner.

A few measurements of the decrease of viscosity upon aging of the dispersion were made during several days' time with and without previous heating or addition of preservative. The viscosity data are presented in Table IV.

Evaluation of the Fractions in Handsheets. The Waring Blendor beater and Weyerhaeuser bleached sulfite stock were used as previously described in Reports 1 and 2. The gums and gum fractions were dispersed in water according to the procedure for determination of the relative viscosity and added to the pulp just before the five minute beating period was started. The beaten pulp was made into 1.5 g. handsheets and conditioned and tested in the customary manner.

Determination of the Specific Rotation of the Fractions. Several attempts were made to determine the specific rotations of the fractions in water, but difficulties were encountered because of the opalescence of the dispersions which scattered the light. Other solvents were tried but without success. Saturated neutral calcium chloride solution dissolved part of the gum when stirred overnight at room temperature, but this dispersion could not be used. Sixty per cent zinc chloride solution dissolved the gum, but the dispersion was milky. Sodium hydroxide solution (0.4%) gave a fairly

good dispersion of the cold water soluble fraction but did not adequately disperse the hot water soluble fraction or the whole purified gum. Several rather crude values were obtained with a one decimeter tube. These are presented in Table III.

### Results and Discussion

The successive extractions of locust bean gum with cold water and the small amount of gum obtained by the subsequent hot water extraction indicated that no large fraction of the gum was insoluble in cold water. However, from this work it became evident that a considerable part of the gum was more readily soluble in cold water than the remaining portion. An arbitrary fractionation procedure was, therefore, decided upon in order to study these products. This developed into a two hour cold water extraction followed by a one hour hot water extraction of the residue. These fractions (designated C.W.S. and H.W.S.) were evaluated in handsheets before and after hydrolysis and some of their physical properties were determined.

Specific Rotations of the Fractions. The determination of the specific rotations of the fractions possessed on principal difficulty-- attainment of complete dispersion of the gum. The sols produced by dispersion in water were apparently quite satisfactory, but in almost all of the cases the beam of polarized light was diffused and scattered to such an extent that only a rough estimate could be made because of the erratic readings of the saccharimeter used. The difficulty was not quite as pronounced with the C.W.S. fraction as with the whole gum and the H.W.S. fraction.

The rotation of the latter was almost impossible to estimate. The crude values obtained in the designated media are given in Table III.

TABLE III

CRUDE SPECIFIC ROTATION VALUES OF THE FRACTIONS  
 AND PURIFIED WHOLE GUM

Fraction	Dispersion Medium	$[\alpha]_D^{23}$ C=0.8
1 C.W.S.	0.8% HCl	+22.2
Same after 24 hrs.	0.8% HCl	+19.75
Same after 48 hrs.	0.8% HCl	+21.8
2 C.W.S.	Water	—
3 C.W.S.	0.4% NaOH	+19.7
Same after heating to 98° C.	0.4% NaOH	+19.7
Same after 72 hrs. at room temp.	0.4% NaOH	+17.8
4 H.W.S.	Boiling water	-10.0 ? - 7.4
5 H.W.S.	0.4% NaOH	—
6 Whole gum	0.4% NaOH	+8.71
Same after 2 days	0.4% NaOH	+7.4

The values for the whole gum differ quite markedly from that given by Lew and Gortner (Archives of Biochemistry 1, 325 (1943)). These investigators found an average initial value in 2.N HCl of +43.0 which fell to a +7.0 upon hydrolysis and approached a final equilibrium value

of about  $+28^{\circ}$ . The different dispersion medium in our experiment may account for the difference, but since the C.W.S. fraction was not greatly different in the two media it would seem that the whole gum should not be greatly affected. The difference may indicate that our products have been appreciably degraded somewhere during the purification or fraction procedures. It is possible that an enzyme which is suspected to be present in the gum has converted the products. At present this remains unsolved. Further attempts should be made to obtain the true specific rotation values of the fractions.

Relative Viscosities of the Arbitrary Fractions. The relative viscosities of the fractions under various conditions are presented in Table IV. From this data it is evident that the fractions differ markedly in relative viscosity when adequately dispersed. The difference was not particularly great when both fractions were dispersed at  $70^{\circ}$  C; the C.W.S. averaged about 20 and the H.W.S. about 25. Heating of these dispersions to boiling or  $95^{\circ}$  C. caused a marked difference to appear. The C.W.S. fraction decreased slightly while the H.W.S. fraction increased to about 36-40. It was evident from variations in successive determinations of the time of flow that the H.W.S. fraction did not fully disperse at  $70^{\circ}$  C. since marked structural viscosity effects were noted. Heating the H.W.S. dispersion to  $95^{\circ}$  C. and cooling to  $30^{\circ}$  C. gave very good checks indicating much better dispersion.

TABLE IV

VARIATION IN RELATIVE VISCOSITY OF THE FRACTIONS UNDER VARIOUS CONDITIONS

Fraction	Dispersion Treatment	Initial	Relative Viscosity After Aging (Days)																
			1	2	3	4	5	6	10	40	47								
1 C.W.S.	Cold dispersed, not heated	19.78	5.88	2.32	1.95	--	1.69	--	--	--	--	--	--	--	--	--	--	--	47
2 C.W.S.	Heated to 76° C.	18.6	18.20	5.41	1.94	--	1.40	--	--	--	--	--	--	--	--	--	--	--	--
3 C.W.S.	Cold dispersed, not heated	19.75	10.4	3.31	--	--	1.42	--	--	--	--	--	--	--	--	--	--	--	--
4 C.W.S.	Heated to 95° C.	18.2	17.7	17.2	--	--	14.9	--	--	--	--	--	--	--	--	--	--	--	--
5 C.W.S.	Heated to 95° C.	22.1	--	--	--	--	--	--	--	--	--	21.8	--	--	--	--	--	--	19.6
6 C.W.S.	Dispersed at 70° C.	21.7	20.7	5.88	--	1.69	--	--	--	--	--	--	--	--	--	--	--	--	--
7 C.W.S.	Cold dispersed + tiny crystal phenol	18.9	15.8	10.3	7.40	--	3.78	--	--	--	--	--	--	--	--	--	--	--	--
8 C.W.S.	Oven dried 105° C.	10.5	10.5	10.2	3.3	--	--	--	--	--	--	--	--	--	--	--	--	--	--
9 H.W.S.	Dispersed at 70° C.	18.7	18.6	18.7	17.2	15.8	--	11.5	--	--	--	--	--	--	--	--	--	--	--
10 H.W.S.	Oven dried 105° C. Dispersed at 70° C.	25.2*	24.6	22.4	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
11 H.W.S.	Dispersed at 70° C. Heated to 95° C.	--	--	41.3**	--	40.2**	--	--	--	--	--	--	--	--	--	--	--	--	--
12 H.W.S.	Heated to 95° C.	36.3	--	--	--	--	--	--	--	--	33.1	--	--	--	--	--	--	--	22.6
13 C.W.S.-E5	Heated to 95° C.	1.56	--	1.53	1.52	--	--	1.48	--	--	--	--	--	--	--	--	--	--	--
14 C.W.S.-E5	Heated to 95° C. + 1 ml. phenol	1.73	--	1.71	1.72	--	--	1.68	--	--	--	1.71	--	--	--	--	--	--	1.71
15 H.W.S.-E5	Heated to 95° C.	1.70	1.68	--	--	1.64	--	--	--	--	--	1.71	--	--	--	--	--	--	--
16 C.W.S.-H10	Heated to 95° C.	1.54	1.54	--	--	--	--	--	--	--	1.50	--	--	--	--	--	--	--	1.46
17 H.V.S.-H10	Heated to 95° C.	1.22	1.26	--	--	--	--	--	--	--	1.26	--	--	--	--	--	--	--	1.23
18 H2O-C.W.S.	Heated to 95° C.	1.43	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
19 H2O-H.V.S.	Heated to 95° C.	1.95	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

\* Very erratic checks were noted because of structural viscosity  
 \*\* Very good checks noted because better dispersed after heating to 95° C.

The C.W.S. fraction was unique because of its rapid fall in viscosity upon aging. Dispersion of the fraction by stirring at room temperature gave sols possessing viscosities of about the same magnitude as those dispersed by heating, but the nonheated sols dropped in viscosity much more rapidly than those which had been heated. This may indicate the presence of an enzyme in the mucilage. Heating would probably tend to inactivate such an enzyme and rapid drops in viscosity of the H.W.S. fraction and heated C.W.S. dispersions would not be expected. This was found to be the case. The addition of one ml. of phenol (melted crystals) per 100 ml. of sol inhibited the viscosity drop, but the addition of a very small crystal (approx. 1 mg.) only slowed the fall in viscosity. (See 7, Table IV). Oven dried fractions (16 hours at 105° C.) when dispersed at 70° C. had lower initial viscosities and showed a much slower drop in viscosity upon aging. While these observations indicate that an enzyme may be present they do not exclude the possibility that micro-organisms from the air and glassware may be causing the fall in viscosity. If an enzyme is responsible, then it is likely that the cold water soluble fraction has been degraded during the two hour extraction period or perhaps this fraction is the product of the enzyme action on the whole gum. Further work is necessary for conclusive evidence.

The viscosity data for the hydrolyzed fractions show that quite extensive degradation had taken place during the five and ten minute periods.

The values of the fractions isolated from the 20 minute hydrolyzed gum compared quite well with those of the five and ten minute fractions which had been hydrolyzed subsequent to extraction. The fact that different times were required to effect hydrolysis in the two experiments may possibly be attributed to a difference in particle size during the conversion.

Evaluation of the Fractions in Handsheets. It is believed that the handsheet data presented in Table V should not be considered as conclusive evidence of the merits of the fractions because they are the average results of ten sheets made at one freeness value—835. A slower stock might not emphasize the differences between the fractions to the same extent. It appears, however, that the values do indicate real trends at freeness 835.

The C.W.S. fraction seemed to be superior to the H.W.S. fraction in burst, fold, and tear values, but when the fractions were hydrolyzed for five minutes the relative values excepting tear were in the same order but closer together and much greater in magnitude. The C.W.S. fraction gave a bursting strength increase of 44.1% and a fold increase of 300%. When this fraction was hydrolyzed for 5 minutes (C.W.S.-H5) an 84% increase in burst and a 400% increase in fold resulted. After a ten minute hydrolysis of the fraction the burst increased 56% and the fold 334% indicating that degradative effects were beginning to decrease the strength properties. At the ten minute hydrolysis time the H.W.S.-H10 fraction was slightly better than the C.W.S.-H10 fraction. It appears, therefore, that the

TABLE V

A HANDSOME COMPARISON OF THE WHOLE GUM WITH THE CONVERTED AND UNCONVERTED FRACTIONS  
(1% of gum on weight of pulp)

Fraction or Gum Used	Basle Weight 25x40/500	Caliper Inch	Bursting Strength Mullen Pts./100 lbs.	Per cent Increase Mullen Fold	MIF Fold	Per cent Increase in Fold	Year Elapsed	Year Factor	Per cent Increase in Year	Flwing Formation	Increase in Formation %	I.P.G. File Number
Blank	46.5	0.0051	11.4	25	3	---	77	1.66	---	---	---	110300
C.W.S.	46.6	0.0050	16.9	36	12	300	114	2.45	58.4	---	---	110301
H.V.S.	45.5	0.0050	13.7	30	7	133	108	2.37	42.8	---	---	110302
C.V.S.-Hydrolysed 10 minutes	46.6	0.0050	18.4	39	13	334	137	2.94	77.2	---	---	110303
H.V.S.-Hydrolysed 10 minutes	46.5	0.0050	18.5	40	14	367	114	2.45	58.4	---	---	110304
Blank	47.6	0.0051	11.9	25	4	---	99	2.08	---	33.0	---	110327
C.V.S.-Hydrolysed 5 minutes	47.0	0.0050	21.4	46	20	400	101	2.15	33.6	41.7	26.4	110328
H.V.S.-Hydrolysed 5 minutes	46.7	0.0050	20.5	44	17	325	112	2.40	35.4	39.7	20.3	110329
Raw Whole Gum	47.3	0.0049	15.3	32	9	125	110	2.33	12.0	47.1	42.2	110423
Purified Raw Whole Gum	47.6	0.0049	15.3	32	9	125	117	2.46	20.1	49.2	49.2	110424
20 minute hydrolysed gum	45.7	0.0049	19.4	40	16	300	133	2.73	31.2	39.0	18.2	110425
Purified 0103-431	45.7	0.0050	19.3	40	16	300	132	2.71	30.3	40.2	21.8	110426
C.V.S. of 0103-431 H2O-0.W.S.	46.7	0.0049	19.2	41	16	300	120	2.57	23.6	39.4	19.4	110427
H.V.S. of 0103-431 H2O-H.V.S.	47.3	0.0049	21.2	45	26	550	127	2.66	28.8	41.5	25.8	110428

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H.W.S. fraction of the gum is better able to withstand the hydrolysis treatment--possibly because it is more highly polymerized. However, this assumption does not seem to be supported by the viscosity data.

On the basis of the data of Table V limited hydrolysis seems to improve the gum fractions and whole gum in so far as burst and fold increases are concerned. Both the raw and purified whole gum gave a 28% increase in bursting strength and a 125% increase in folding endurance. When this whole gum was hydrolyzed for 20 minutes (G103-431) both the crude and purified products gave a 60% increase in burst and a 300% increase in fold. The crude hydrolyzed gum was fractionated into cold water soluble and hot water soluble fractions similarly to the raw gum. These were designated as H2O-C.W.S. and H2O-H.W.S. fractions meaning that they were hydrolyzed first and then fractionated. The H2O-C.W.S. fraction was equal to the whole hydrolyzed gum in burst and fold and about equal to it in tear value. The increases in tear values upon addition of the fractions to the pulp are believed to be characteristic of the pulp at the low freeness value employed for this work. The somewhat erratic values obtained in some cases remain unexplained.

Improvement in formation value was greatest with the whole unconverted gum according to the data available. The raw gum gave a 42.2% increase and the corresponding purified product gave a 49.2% increase.

Future Work

These fractions are to be used as tubsizes, and the results will be given in a subsequent report. Further attempts to measure the optical rotations of the fractions will be made.

Summary

1. Locust bean and Guar gums were exhaustively extracted with cold water followed by hot water. It was found that no large portion of either of the mucilages was insoluble in cold water, but a considerable part was more readily soluble than the remaining fraction.
2. An arbitrary cold water and hot water fractionation procedure was developed and some properties of the fractions were determined.
3. The viscosity data of the cold water soluble fraction indicate the possibility of an enzyme being present in the mucilage. The viscosities of the cold and hot water soluble fractions differ widely when thoroughly dispersed.
4. Attempts were made to determine the specific rotations of the fractions. Opalescence of the solutions made this quite difficult, but a few rough values were obtained.
5. The fractions were hydrolyzed similarly to the procedure used on the raw whole gum, and these products were incorporated into handsheets. The hydrolyzed fractions gave considerably higher bursting strength and folding endurance than the nonhydrolyzed fractions.

jws/acj

# PROJECT REPORT FORM

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## VISCOSITY AND PAPER TESTS ON SAMPLES OF GUAR GUM OF THE TYPES EXPECTED TO BE MADE FOR COMMERCIAL USAGE

### Procedures:

Two new guar gums, G4-2's, were received from G.M.I. on 9-2-43 and were described as (a) old G4-2 No. R22602 and (b) new G4-2 No. R23267 "in which the enzyme had been inactivated." When put into water, the old guar G4-2 No. R22602 was apparently more viscous than the original old G4-2 sample No. R22539 which showed up so poorly in previous work. Its apparent viscosity was also greater than the new G4-2 No. R23267 immediately after dispersing in cold water. The latter sample had only a slight trace of the characteristic "grassy" odor but was slightly darker in color (grey). In addition, a sample of finely ground yellow G4 guar gum No. R22603, similar to samples Nos. R22376-R22378 previously described, was received.

Viscosity tests on these cooked gums were made at 30.0° C. in an oswald pipette (water at 30.0° C. runs 5.25 seconds). Eight hundred grams of 0.50% gum solids (containing 11% moisture, or in other words, 3.56 grams oven-dry are equivalent to 4.0 grams containing 11% moisture) were prepared in each case by dusting the gums into 80° C. distilled water, cooking at 80° C. for 10 minutes and cooling rapidly in a water bath. The cooked gums were run in the pipette at .5, .4, .3, .2, and .1% solids on a 11% moisture basis. For comparison, the viscosity of a coarse yellow guar G4 gum was also measured. The sample used was "yellow G4 No. R22378 over 5400" which was a

part of the sample sent to several mills for trials and which gave very favorable results. After cooking this sample, the suspended matter was too coarse for the pipette so the viscosity was measured after the following two treatments (a) centrifuged after cooking and the clear top liquor run into the pipette, the solids being based on the original solids and not on those actually present in the decanted liquor, and (b) dispersed cold in the Waring Blendor at high density followed by cooking at 80° C. The results are given in terms of relative viscosity in Table IA.

TABLE IA  
 RELATIVE VISCOSITY

<u>Percent Solids</u>	<u>.5</u>	<u>.4</u>	<u>.3</u>	<u>.2</u>	<u>.1%</u>
Cooked R22602 (old G4-2)	7.905	5.048	3.181	2.076	1.429
Cooked R23267 (New G4-2)	6.019	4.095	2.724	1.867	1.352
Cooked R22603 (Fine, Yellow G4)	15.467	8.686	4.800	2.686	1.581
Cooked and centrifuged R22378 (coarse, yellow G4)	27.371	13.867	6.762	3.371	1.733
Cooked and homogenized R22378 (coarse, yellow G4)	42.286	19.410	8.667	3.848	1.848

Compare these viscosity results with those of other preparations previously reported. When these viscosities are plotted on semi-log graph paper with the relative viscosities as ordinates (the log scale) and the concentrations as abscissa, straight lines result.

Sheet tests followed the standard procedure for this project. Additions of the gums were made at 1/2, 1, and 2% solids based on the weight

of the oven-dry fiber, with the weights of the gums calculated to an 11.0% moisture content basis. In addition, a series of experiments were made in which the gums were added dry to the beater at 1-1/2% solids and beaten for samples at two beating intervals.

For the regular tests, each of the samples including the commercial sample of locust bean gum were introduced in both the cooked and uncooked conditions. Cooking was carried out by the same procedure as described for the viscosity tests above. The two G4-2 samples were tested, in addition, after treatment with room temperature distilled water for 18 hours to determine the differences in the actions of the self-contained enzymes. During this aging period the suspensions were kept in glass beakers covered with watch glasses. The sheet tests: formation, bursting strength, and wet tensile strength were run on the prepared papers, the latter test after dipping the sheet in 1.0% sodium tetraborate and redrying. The results for the first part of this work are given in Tables I, II, and III with a summary in Table IV. Table I shows the resultant formation numbers of sheets prepared with 1/2, 1, and 2% of the cooked and uncooked gums. It is clear from these data that none of the gums tested are nearly as effective as locust bean gum as a fiber deflocculant. The enzyme inactivated and cold water soaked guar No. R23267 appears to give the best results of the samples other than the control samples, although the cooked G4-2 No. R22602 (the product which G.M.I. intends to make commercially) and the cooked No. R23267, also rank quite high. Adding these samples uncooked decreases their effectiveness as deflocculating agents. This is in line

with the theory that these dry milled gums do not reach their maximum hydrous characteristics unless cooked and that prolonged standing in cold water of a nonenzyme deactivated gum will degrade it and ruin its hydrous properties. The results then follow in direct proportions, the viscosities of the respective gum suspensions; a cold water suspension of the R22602 was found to have, immediately after dispersing, an apparent viscosity higher than that of the same milled sample, but enzyme deactivated, No. R23267. However, on allowing both to stand for 18 hours at room temperature, the R22602 became thinner than originally due to possible enzyme action, while the R23267 became more hydrous as indicated by its increase in apparent viscosity as well as by its greater fiber deflocculating power. The fact remains, however, that all of these samples are low in viscosity when compared with locust bean gum and are correspondingly deficient in their fiber deflocculating properties.

Table II shows the bursting strength results. Here the gums are more nearly equal to the results for locust bean gum, in fact when allowance is made for the 20% inert material in these dry milled products, some of them are more effective than uncooked locust bean gum. The cooked G4-2's No. R22602 and No. R23267 as well as the uncooked fine yellow G4 No. R22603 and the uncooked G4-2 No. R22602 appear to be quite good. None of the samples equals the effectiveness of cooked locust bean gum. Prolonged beating will, of course, narrow the spread between the samples as well as lower the percentage increases in strength over the blanks, but it has been found both in previous work here as well as in mill practice that those

TABLE I

THWING FORMATION NUMBERS OF SHEETS TREATED WITH THE COOKED AND UNCOOKED GUMS, PLOTTED IN ORDER OF EFFECTIVENESS AT THE TWO PERCENT CONCENTRATION

	2% Addition	% Increase over the blank at 2% addition	1% Addition	% Increase over the blank at 1% addition	1/2% Addition	% Increase over the blank at 1/2% addition
Cooked locust bean gum	43.9	73.52%	44.4	75.49%	39.6	56.52%
Uncooked locust bean gum	39.4	55.73	37.8	49.41	36.7	45.06
Cold water soaked 18 hr. R23267 (new G4-2)	38.2	50.99	35.0	38.34	31.4	24.11
Uncooked guar R22603 (fine, yellow G-4)	36.0	42.29	32.3	27.67	31.9	26.09
Cooked guar R22602 (old G4-2)	35.8	41.50	34.7	37.15	32.8	29.64
Cooked guar R23267 (new G4-2)	34.8	37.55	35.0	38.34	31.9	26.09
Uncooked guar R23267 (new G4-2)	34.8	37.55	32.6	28.85	31.6	24.90
Cooked guar R22603 (fine, yellow G-4)	34.7	37.15	32.5	28.46	33.7	33.20
Cold water soaked 18 hr. R22602 (old G4-2)	34.5	36.36	33.0	30.43	34.3	35.57
Uncooked guar R22602 (old G4-2)	34.5	36.36	32.3	27.66	29.4	16.21
Untreated sheet	25.3					

TABLE II

BURSTING STRENGTH IN POINTS OF SHEETS TREATED WITH THE COOKED  
AND UNCOOKED GUMS, PLOTTED IN ORDER OF EFFECTIVENESS AT THE  
TWO PERCENT CONCENTRATION

	2% Addition	% Increase over the blank at 2% addition	1% Addition	% Increase over the blank at 1% addition	1/2% Addition	% Increase over the blank at 1/2% addition
Cooked locust bean gum	34.0	88.89%	28.7	59.44%	26.9	49.44%
Uncooked locust bean gum	29.9	66.11	26.9	49.44	25.1	39.44
Cooked guar R22602 (old G4-2)	29.7	65.00	27.3	51.67	22.6	25.56
Uncooked guar R22603 (fine, yellow G-4)	29.1	61.67	26.2	45.56	25.4	41.11
Cooked guar R23267 (new G4-2)	28.9	60.56	27.4	53.22	24.3	35.00
Cooked guar R22603 (fine, yellow G4)	28.4	57.78	25.5	41.67	24.2	34.44
Uncooked guar R22602 (old G4-2)	27.9	55.00	26.2	45.56	24.2	34.44
Cold water soaked 18 hr. R23267 (new G4-2)	27.7	53.89	25.4	41.11	23.2	28.89
Uncooked guar R23267 (new G4-2)	27.1	50.56	24.0	33.33	21.7	20.56
Cold water soaked 18 hr. R22602 (old G4-2)	26.1	45.00	24.5	36.11	22.0	22.22
Untreated sheet	18.0					

TABLE III

WET STRENGTH IN UNITS PER 15 MM. STRIP OF SHEETS TREATED WITH  
THE COOKED AND UNCOOKED GUMS, AND 1% BORAX DIPPING SOLUTION

	2% Addition	% Increase over the blank at 2% addition	1% Addition	% Increase over the blank at 1% addition	1/2% Addition	% Increase over the blank at 1/2% addition
Cooked locust bean gum	12.78	526.5%	10.12	396.1%	7.96	290.2%
Uncooked locust bean gum	9.80	380.4	8.50	316.7	6.22	204.9
Uncooked guar R22603 (fine, yellow G4)	9.76	378.4	7.54	269.6	6.60	223.5
Cooked guar R22602 (old G4-2)	9.68	374.5	7.62	273.5	5.08	149.0
Cooked guar R22603 (fine, yellow G4)	9.36	358.8	8.24	303.9	6.72	229.4
Cooked guar R23267 (new G4-2)	8.34	308.8	7.26	255.9	5.70	179.4
Uncooked guar R22602 (old G4-2)	8.28	305.9	7.00	243.1	4.98	144.1
Cold water soaked 18 hr. R23267 (new G4-2)	8.18	301.0	6.36	211.8	5.56	172.5
Uncooked guar R23267 (new G4-2)	7.46	265.7	5.90	189.2	5.36	162.7
Cold water soaked 18 hr. R22602 (old G4-2)	6.58	222.5	6.50	218.6	4.84	137.3
Untreated sheets	2.04					

TABLE IV

TEST RESULTS (EXTRAPOLATED FROM SMOOTHED CURVES) AT 1.25% CONCENTRATION  
TO COMPENSATE FOR THE 20% INERT MATERIAL IN THE MILLED GUMS,  
COMPARED WITH THE RESULTS FOR LOCUST BEAN GUM AT 1.0%

	<u>Thwing</u> <u>Formation Numbers</u>			<u>Bursting Strength</u>			<u>Wet</u> <u>Tensile Strength</u>		
	<u>Formation</u> <u>Number</u>	<u>Rank</u>	<u>% of result</u> <u>for uncooked</u> <u>Locust B. Gum</u>	<u>Points</u>	<u>Rank</u>	<u>% of result</u> <u>for uncooked</u> <u>Locust B. Gum</u>	<u>Units</u>	<u>Rank</u>	<u>% of result</u> <u>for uncooked</u> <u>Locust B. Gum</u>
Uncooked guar R22602 (old G4-2)	32.60	8	86.0	26.80	4	98.5	7.2	5	86.7
Uncooked guar R23267 (new G4-2)	33.45	7	88.3	24.85	7	91.3	6.6	7	79.5
Uncooked locust bean gum	37.90			27.22			8.3		
Cooked guar R22602 (old G4-2)	35.00	2	92.3	27.46	3	100.9	8.2	3	98.8
Cooked guar R23267 (new G4-2)	34.40	3	90.8	27.65	1	101.6	7.6	4	91.6
Cooked locust bean gum	42.20		111.3	29.46		108.2	10.3		124.1
Uncooked guar R22603 (fine, yellow G4)	33.90	6	89.4	27.55	2	101.2	8.6	2	103.6
Cooked guar R22603 (fine, yellow G4)	34.00	5	89.7	26.50	5	97.4	8.7	1	104.8
Cold water soaked 18 hr. R22602 (old G4-2)	34.05	4	89.8	24.80	8	91.1	6.4	8	77.1
Cold water soaked 18 hr. R23267 (new G4-2)	35.65	1	94.1	26.05	6	95.7	7.1	6	85.5

which show up well on lightly hydrated pulps, usually hold their same relative positions on increased heating.

Table III shows the wet tensile strength in units (one unit equals 0.207 pounds) of 15mm. strips of sheets treated with the cooked and uncooked gums and after 1.0% borax dippings. Note the marked increase in strength of even the weakest gum at its lowest concentration over the blank sheet which was also given the borax treatment. None of the samples is equal to either cooked or uncooked locust bean gum unless a correction is made for the 20% inert materials in which case the fine yellow guar gum No. R22603 slightly exceeds the result for uncooked locust bean gum.

Table IV is a summation of the above described test results. These data were obtained as follows: the test results were plotted on graph paper for the three physical tests and smooth curves drawn from the four points: 0, 1/2, 1, and 2% concentrations. From these curves were read the test results at a concentration of 1.25% for the samples prepared by G.M.I. and for 1.0% for the locust bean gum. This was done to put the samples on a more equal gum content basis since the dry milled samples were first described on containing about 20% of nonmannogalactan solids. Since then it was found that the nonmannogalactan content, especially of the G4-2 samples, was somewhat higher but since locust bean gum itself contains all the way from 6 to 10% of these inert solids, the 20% figure was adopted as being about the correct figure for an equal mannogalactan content comparison with locust bean gum. The second column under each heading indicates the relative

rank for each gum and for each of the methods of preparation, while the third column under each heading shows the percent of effectiveness considering the results for uncooked locust bean gum as unity. A study of the table shows that in general the G.M.I. gums are deficient in fiber deflocculating powers, while as dry or wet adhesives they are more equal, and superior in some cases to the uncooked standard of comparison. Cooking the locust bean gum increases its effectiveness in all three respects to a point where it is definitely superior to any of the other samples regardless of the method of addition or the correction for inert materials. The deficiencies in the latter two properties by these guar samples are not as serious from a commercial viewpoint as would appear from these results. Other factors such as (a) steady source of supply, (b) finer particle size allowing direct addition to the pulp furnish without cooking, and (c) possibly lower price; may outweigh these intrinsic differences between the old and the newly developed gums.

Tables V, VI, and VII show the test results of dry gum additions directly to the beater where the beating interval was varied. The sheets prepared from the yellow guar G4 over 8xx No. R22377 were very spotted from the undispersed gum, especially at the lower beating interval. These spots were apparently so close together and evenly spaced that they did not register on the formation tester as evidenced from the high formation readings obtained.

Although the probable error is higher here than in the tests described above, the same general conclusions can be drawn i.e. (1) the guar gums are not equal to uncooked locust bean gum as deflocculating agents,

(2) they approach equality with locust bean gum as dry and wet adhesives under the conditions described when corrections are made for inert materials present, (3) they have other desirable qualities which locust bean gum does not possess.

TEWING FORMATION NUMBERS OF SHEETS PREPARED BY ADDITIONS  
OF 1-1/2% DRY GUMS DIRECTLY TO BEATER

	10 Minute Beating Interval		30 Minute Beating Interval	
	Formation Number	% of Result for Locust Bean Gum	Formation Number	% of Result for Locust Bean Gum
Locust Bean Gum	42.6		42.6	
Guar R23267 (new G4-2)	38.1	89.4	41.6	97.5
Guar R22377 (yellow G4 over 8xx)	37.7	88.5	42.9	100.6
Guar R22603 (fine yellow G4)	37.2	87.2	40.7	95.5
Guar R22602 (old G4-2)	35.7	83.8	40.5	95.0
Pulp Only	27.1	63.6	30.7	72.0

TABLE VI

BURSTING STRENGTH IN POINTS OF SHEETS PREPARED BY  
ADDITIONS OF 1-1/2% DRY GUMS DIRECTLY TO BEATER

	10 Minute Beating Interval		30 Minute Beating Interval	
	Points	% of Result for Locust Bean Gum	Points	% of Result for Locust Bean Gum
Guar R22603 (fine yellow G4)	30.6	101.0	48.7	93.7
Locust Bean Gum	30.3		52.0	
Guar R22602 (old G4-2)	29.3	96.8	48.8	93.8
Guar R22377 (yellow G4 over 8xx)	29.1	96.1	49.7	95.6
Guar R23267 (new G4-2)	28.7	94.8	49.6	95.4
Pulp Only	18.4	60.7	38.6	74.2

TABLE VII

WET TENSILE STRENGTH OF SHEETS PREPARED BY ADDITIONS OF  
1-1/2% DRY GUMS TO BEATER AND GIVEN BORAX DIPPINGS

	10 Minute Beating Interval		30 Minute Beating Interval	
	Wet Tensile Units Per 15 mm. Strip	% of Result for Locust Bean Gum	Wet Tensile Units Per 15 mm. Strip	% of Result for Locust Bean Gum
Locust Bean Gum	11.3		15.9	
Guar R22603 (fine, yellow G <sup>4</sup> )	10.4	92.2	18.3	115.1
Guar R22377 (yellow G <sup>4</sup> over 8xx)	9.8	86.8	16.9	106.3
Guar R22602 (old G <sup>4</sup> -2)	9.4	83.2	14.1	88.7
Guar R23267 (new G <sup>4</sup> -2)	8.9	78.9	16.9	106.3
Pulp Only	1.7	15.0	3.0	18.9

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PROJECT NO. 849  
COOPERATOR Institute  
REPORT NO. 2  
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SIGNED John W. Swanson  
John W. Swanson

## IMPROVEMENT OF FORMATION AND TEAR VALUES THROUGH BEATER ADDITION OF HYDROLYZED LOCUST BEAN GUMS

### Introduction

The formation and tear data presented in Report No. 1 were unsuitable for an adequate evaluation of these properties of the hydrolyzed gums. This report presents some additional experimental data on these properties.

### Experimental

The Valley beater used in Report No. 1 did not give a pulp of suitable character for evaluation of the formation and tear characteristics of the gums and a Waring Blendor was tried as a beater. At first, it was believed that this machine would disperse the pulp with very little actual beating, but it was soon learned that beating was accomplished.

Attempts to make a blank sheet possessing a low formation value from Weyerhaeuser bleached sulfite were unsuccessful. It was decided that a harder, longer fibered stock should be used and Duracel bleached kraft was selected. This pulp did not give a particularly low formation value, but it was sufficient to show decided improvement upon addition of the hydrolyzed gums. A few preliminary experiments with Waring Blendor beating of this pulp were first tried in order to find out if the beating time seriously affected the degree of formation. These experiments are summarized in Table I. From these data it was decided that five minutes beating time would be used.

TABLE I  
FORMATION CHANGES OF DURACEL BLEACHED SULFITE ON BEATING

Beating Time Minutes	Thwing Formation
1	32.6
3	35.4
5	36.9
5	37.2
10	37.0
15	36.5

#### Beating Procedure

Ten grams of the pulp (O.D. basis) were placed in a Waring Blender and 714 ml. of water and 0.1 g. of the air dried converted gum were added in succession. The mixture was allowed to soak for five minutes and then the beater was turned on (low speed) for exactly five minutes. The pulp was diluted to 2000 ml. (0.5%) and made into 1.5 g. handsheets on a Valley mold in the usual manner. For purposes of comparison the 5 minute gum was also added to the pulp from a water mixture before and after heating to complete the dispersion.

#### Results and Discussion

The formation data (Table II) show quite clearly that the hydrolyzed gums increased the formation quality 31.4-43.8%. There was a gradual improvement to the ten minute gum and then a rapid falling off as the degree of hydrolysis increased. The formation of the 5-minute gum dispersed in cold water alone was poorer than when heated with water for a more complete dispersion.

The tear values also show an increase to the 10-minute gum and then begin to decrease. No value was obtained for the 20-minute gum because all of the sheets gave bad tears when tested. A higher tearing resistance was obtained with addition of the heated gum than

TABLE II

THE FORMATION AND TEAR VALUES OF HANDSHEETS UPON ADDITION  
 OF HYDROLYZED LOCUST BEAN GUMS

Gum Added	Basis Weight 25x40/500	Thwing Forma- tion	Per cent Increase in Forma- tion	Elmen- dorf Tear g/sheet	Tear Factor	Per cent Increase in Tear	File No.
Blank	47.9	34.7		106	2.21		110457
Raw gum	46.7	49.2	41.8	133	2.85	29.0	110458
5 minute gum	48.1	49.5	42.6	143	2.97	33.4	110459
10 minute gum	50.2	49.7	43.8	174	3.47	57.1	110460
20 minute gum	48.9	47.9	38.0	No good tears out of 7 tests			110461
30 minute gum	49.8	45.6	31.4	160	3.21	45.3	110462
Blank	47.3	37.8		105	2.22		110584
5 minute gum added from cold water mixture	47.6	49	29.7	141	2.96	33.3	110584
5 minute gum added after heat- ing with water	47.8	51.2	38.1	152	3.18	43.3	110584

with that of the cold dispersed gum. The tear values reported here must not be taken as necessarily indicative of mill operation. It should be remembered that the tear values obtained from pulp beaten in the Valley laboratory beater were decreased considerably upon addition of the converted gums. (See Report No. 1) Further experimentation with the Waring Blender has indicated that a simultaneous improvement of burst, fold and tear values may be obtained at least up to a certain beating degree. This may be due to a different kind of fiber produced by this type of beater. Therefore, the tear value data cannot be interpreted as the result of gum addition alone but must be interpreted as a combination of the unusual fiber and the gum. The factors contributing to these results are being studied further.

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Examination of semi-commercial mucilage samples prepared by General Mills, Inc. and of other manno-galactan types of natural plant mucilages for their properties in paper as fiber deflocculents and strengthening materials.

## Preparation of the gums:-

Five mucilage samples prepared by a dry milling process were first received from General Mills, Inc. Among these were the first of the "rolled" products obtained, products which were put through flaking rolls usually in an alkaline condition to break down the endosperm cell walls and thus allow a much finer dispersion when wetted than would otherwise be possible. The two unrolled samples were "Honey locust bean gum through 100 mesh" and "Flame tree seeds, germ removed, over 60 mesh." The latter sample was of a dark color and contained large proportions of the seed coat material while the honey locust bean gum was very light colored and had the appearance of a good grade of imported locust bean gum (*Ceretonia siliqua*). The three alkaline rolled samples were guar endosperms ground to pass various mesh sizes. These were all olive drab in color. The mesh sizes are given in the table of data, Table I, below.

The dispersion procedures used on these gums were similar to those given in previous reports i.e., 3.0 g. (A.D. wt.) powdered gum dusted into 297 g. of cold distilled water. Stirred for two hours continuously with a glass propellor, then passed through the hand operated homogenizer at 1% solids, two passes, diluted to double the volume and filtered through a tared wire sieve having 50 meshes per inch in the weft direction and 72.5 meshes per inch in the warp direction (referred to as 80 mesh) in a 7 cm. Buchner funnel over suction, dried at 105° c. and weighed. Before application of the dispersed gum, (that portion which passed the wire sieve) it was measured for its solids content by weighing portions of it into tared weighing bottles, drying to constant weight at 105° C. and then converting the oven-dry weights of solids to the air-dry basis by dividing by 0.89 (11% moisture). The per cent added to pulp is based on these latter air-dry figures and on the weight of the oven-dry pulp. The apparent amount of material from each of these three guar gum samples retained on the wires was very low compared with that obtained from other dry milled guar endosperm samples previously received. The other samples mentioned as well as a regular sample of imported locust bean gum were also dispersed by the procedure described. The flame tree seed gum was difficult to put through the homogenizer because of the comparatively large size of extremely hard particles. One pass through the homogenizer was all that was made and then at a light pressure setting to avoid frequent clogging. The

color of the filtered suspension was very dark. The amounts retained on the 50 x 72.5 mesh sieve after the water dispersing procedures are shown in Table I.

TABLE I

Gum	Per cent retained on wire, air-dry basis
"Rolled guar gum, alkaline, through 80 mesh"	2.413
"Rolled guar gum, alkaline, through 60 mesh on 80"	5.54
"Rolled guar gum, alkaline, through 42 mesh on 60"	7.28
"Honey locust bean gum, through 100 mesh"	9.90
"Flame tree seeds, germ removed, over 60 mesh"	62.60
Imported locust bean gum	5.37

The pH of the dispersion made from the "rolled guar gum, alkaline, through 80 mesh," was measured. It was found to be pH 8.14. Its color at about 0.5% solids was olive green but on adding 10 drops of a 10% solution of aluminum sulfate to 500 grams, the pH dropped to pH 4.45 and the color turned a pale yellow. This might indicate that the alkaline materials would not greatly interfere with the paper color if the pH of the stock were about that of ordinary rosin sized paper.

The flame tree seed mucilage mentioned was not applied to paper because of the very large amounts of undispersible matter and because of the color, these two properties making the gum probably unsuitable as a commercially adaptable material. The others were applied and the papers tested for formation, bursting strength and wet tensile strength by procedures previously described. The sheets for wet strength testing were first subjected to 1% borax dippings and then redried before conditioning and testing. The test results are shown in Tables II, III, and IV. It can be seen that all of the samples gave positive results to a high degree. The honey locust bean gum is superior in all departments to the other three samples but the differences are slight. Those portions of the rolled alkaline guar gum passing the finer mesh sieves seem to be slightly more effective than the coarser material.

TABLE II

## FORMATION NUMBERS (THWING) OF SHEETS

## TREATED WITH GUMS AND MUCILAGES

Gum Used, Semi-Commercial Sample prepared by General Mills, Inc., dispersibles added only.	3% Addition	Rank of Effectiveness at 3% addition	4% Addition	Rank of Effectiveness at 4% addition	2% Addition	Rank of Effectiveness at 2% addition
"Honey Locust Bean Gum, through 100 Mesh"	44.0	1	43.2	1	41.7	1
"Rolled Guar Gum, Alkaline, through 80 Mesh"	43.5	2	40.9	3	39.7	2
"Rolled Guar Gum, Alkaline, through 60 Mesh on 80"	43.1	3	40.8	4	37.7	4
"Rolled Guar Gum, Alkaline, through 42 Mesh on 60"	42.4	4	42.1	2	38.7	3
Blank	29.4					

TABLE III

BURSTING STRENGTH OF SHEETS TREATED WITH  
THE GUMS, IN POUNDS PER SQUARE INCH

Gum Used, Semi-Commercial Sample prepared by General Mills, Inc., dispersible portion added only.	8% Addition	Rank of Effectiveness at 8% addition	4% Addition	Rank of Effectiveness at 4% addition	2% Addition	Rank of Effectiveness at 2% addition
"Honey Locust Bean Gum, through 100 Mesh"	34.9	1	29.3	4	29.8	2
"Rolled Guar Gum, Alkaline, through 60 Mesh on 80"	34.6	2	31.3	2	30.1	1
"Rolled Guar Gum, Alkaline, through 42 Mesh on 60"	34.6	3	30.7	3	28.3	3
"Rolled Guar Gum, Alkaline, through 30 Mesh"	33.3	4	31.9	1	28.0	4
Blank	15.6					

TABLE IV

WET STRENGTHS OF SHEETS TREATED WITH THE  
GUMS AND BORAX, IN WET TENSILE UNITS\*

Gum Used, Semi-Commercial Sample prepared by General Mills, Inc., dispersible portion added only.	8% Addition	Rank of Effectiveness at 8% addition	4% Addition	Rank of Effectiveness at 4% addition	2% Addition	Rank of Effectiveness at 2% addition
"Honey Locust Bean Gum, through 100 Mesh"	14.1	1	11.4	2	9.9	1
"Rolled Guar Gum, Alkaline, through 80 Mesh"	12.7	2	11.5	1	8.2	4
"Rolled Guar Gum, Alkaline, through 60 Mesh on 80"	12.3	3	11.3	3	9.3	2
"Rolled Guar Gum, Alkaline, through 42 Mesh on 60"	11.9	4	10.7	4	9.0	3
Blank	2.1					

\*One wet tensile unit is equivalent to 0.207 pounds. Tests run on 15 mm. strip at rate of loading of two inches per minute.

Eight other milled mucilage samples of various grades and from five different species were received from General Mills, Inc. All were put through water dispersion procedures for measurements of the proportions of coarse and unusable components. Five of them were applied to paper by the same procedure as used for those samples applied above.

The samples received and the amounts of each of them retained on the 50 x 72.5 mesh sieve after the dispersion treatment either in cold or hot water, are listed in Table V, below:

TABLE V

Sample Description	Per cent retained on standard wire the dispersion procedure, A. D. basis	
	Cold Water	80°C. Water
"Honey locust bean gum, alkaline, through 60 mesh, #R21876"	8.04	1.01
"Honey locust bean gum, alkaline, through 60 mesh, #R21880"	17.5	1.32
"Cassia tora gum, through 60 mesh, low grade gum, #R21879"	67.9	60.7
"Palo verde gum, 12-10-42" by Gen. Mills, Inc.	9.14	
"Flame tree seed gum, through 8xx, alkaline, #R21875"	67.2	42.9
"Flame tree seed gum, through 60 mesh, #R21890"	82.2	56.2
"Cassia tora gum, 2-5-43, #R22066"		72.0
"Mesquite bean gum," by Gen. Mills, Inc.		1.42

The two honey locust bean gums, the palo verde gum, the mesquite bean gum, and the flame tree seed gum No. R21875, were the only ones of these samples applied for tests in paper. The other three samples, i.e., the cassia tora gums and the other flame tree seed gum were considered too insoluble for any practical use in paper under normal conditions. The sheet test results are given in the accompanying Tables VII, VIII and IX. Of course, the undispersible (by this procedure) material could be dispersed for all of it to pass the wire sieve by vigorous and prolonged mechanical treatment, but it had been found that this difficult-to-disperse portion is

the least reactive fraction of the gums for our purposes and contains most of the unwanted color pigments. Then too, the costs of this mechanical treatment to a paper mill, would be prohibitively expensive.

#### Experiments on Soy Bean Hulls:-

A mucilage was first prepared from the hulls for the purpose of examining its properties after condensing it to a high solids content. A 95°C. extract of the hulls was made as follows: 150 g. of air-dry and ground soy bean hulls were mixed with 1500 g. water and heated on a boiling water bath with continuous stirring for one hour. It was then filtered while hot on a steam jacketed Buchner funnel through a 72.5 mesh wire sieve. A second extraction of the hulls was made with 750 g. water following the same procedure. The filtrates were combined, heated again to 95°C. on a water bath, and filtered through two layers of cloth toweling on the wire in the steam heated Buchner funnel over suction. Much was retained on the cloth and the filtrate was passed through twice, the second time through the built up filter cake to remove the fines. The cake was finally pressed under a rubber dam. The resultant mucilage suspension was of a brownish tint but was stable as it showed no sediment on prolonged standing. It was stored in a refrigerator for 40 hours before concentrating. The concentration was carried out in a liter distillation flask heated in a water bath. It was heated under vacuum to about 60°C. In about 20 hours, the mucilage was evaporated to 200 g. The concentrate was of a syrupy consistency at 60°C. It was placed in the hand operated homogenizer and given one pass to reduce the lumpy semi-dry material which had clung to the side of the flask and was in the suspension. Solids content measurements averaged 8.18% air-dry solids. This calculates to a 10.3% yield based on the original air-dry weight of hulls, a result which is low because some of the mucilage was lost in transfer and some by the fine filtering through the built-up filter cake. These losses would not be obtained in commercial practice so the expected yield could be much higher. This mucilage was applied to paper as an internal size by the procedure employed on the other mucilages, above. See test results in the accompanying Tables VII, VIII, and IX.

Since shipment of soy hull mucilage would be expensive because of the high water content and since concentrations either by heat or by alcohol precipitation to avoid shipping large quantities of water, are costly processes, another possible means of achieving the desired conditions was tried. This was done by freezing and thawing a water suspension of the mucilage to induce self precipitation of an otherwise stable hydrophylic sol. A 95°C. extract was prepared as above. Filtration was carried on by a like procedure to give a stable and viscous homogeneous suspension. The yield was calculated to be 14.3% air-dry solids based on the original air-dry weight of the hulls. One liter of the mucilage containing slightly over 1% solids was placed in an aluminum beaker, sealed with a rubber dam and placed outside over night to freeze (average temperature 15°F). It was then removed from the cold and thawed. The thawed mucilage showed a stringy precipitate of large volume

indicating some change in the previously homogeneous sol but attempts to collect it on coarse filter paper over suction were unsuccessful because of the rapid clogging of the filter. Concentration by centrifuging was deemed possible but was not tried. The frozen and thawed material was not tested in paper, but examination of its physico-chemical properties by this method and comparing it with its parent material might prove interesting.

Dissolving experiments (viscose) were made on the by-product of the soy bean hull mucilage extraction i.e., the hot water extracted hulls. Two experiments were carried out; one in which the by-product hull material was considered as 100% alpha cellulose (which of course it is not), and one in which it was considered as 80% alpha (which of course it is not). The standard viscose procedure was used except for a few slight variations in aging temperatures. General procedure: 14 g. of air-dry hulls were treated with 210 cc. of 17.5% NaOH solution for 1 hour at 20°C. in a 500 cc. erlenmeyer. Filtered in a small Buchner funnel on an 80 mesh wire, the filter cake removed and pressed between blotting paper in a sheet press to expel excess caustic, to three times its original weight. The cake was then aged out of contact with air for 3 days at about 20°C. 5.6 g. of CS<sub>2</sub> were added and then enough NaOH and water to get a final mixture containing 7% pulp and 6% NaOH. It was then filtered through cloth in a funnel over suction. This was a slow process and some was retained on the cloth, but the high vacuum degassed the filtrate. The samples were aged in the dark for the required length of time (for cellulose) and forced under pressure through a fine glass capillary into a standard viscose spinning bath. The exudation did not cohere into a fiber in either case but fell rather as a moderately loose precipitate. Experiments to find more suitable spinning conditions were not made.

A multi-stage bleaching experiment was also made on the by-product water-extracted soy bean hulls. The hulls, which had been previously extracted (for the mucilage) with 95°C. water and air-dried, were treated with 8% NaOH solution to remove other hemicelluloses and incrusting substances not removed by the neutral hot water. This was done at 8% pulp density with 8% NaOH based on the weight of the hulls and at room temperature. 45 g. of air-dry hulls were treated and in a total weight of 503 g. The caustic was in contact with the hulls for one hour. The material was then washed on a wire in a Buchner funnel with cold water until the wash water became clear. Two doses of chlorine were then given each of about 3.5% chlorine on the original weight of hulls with plain water washings after each stage. The first chlorine exhausted rapidly even though the temperature did not go over 15°C. but the second did not exhaust until heated at 35°C. in a water bath for over one hour. Extraction with about 1% NaOH followed, by treating the washed material with the caustic at 10% pulp density at room temperature for two hours. It was then drained and washed with cold water. The color of the residue was still quite dark so another light chlorination stage was added. About 1 1/2% chlorine, based on the original weight of hulls, were added and exhausted as described above. This chlorinated and caustic extracted residue was then given a hypochlorite bleach of approximately 2.5%. This stage was

done at 10% density and at 40°C. At the end of 1 1/2 hours under these conditions, the bleach was not entirely exhausted as indicated by starch-KI paper but it was washed with large quantities of distilled water, pressed and air dried. Apparent color: very light buff but darker than average printing paper. The yield was 36 g. air dry or 80% of the original weight. (Possible uses: dissolving pulp, plastics, paper filler?)

Mucilage extractions from soy bean hulls were next attempted with water at various elevated temperatures and under various conditions. It had previously been shown on this project that higher temperature extractions gave progressively higher yields of more reactive mucilage. That is, the highest temperature extract which could be obtained at atmospheric pressure by indirect heating, 95°C. to 98°C., were the most potent for the purposes here intended. This indicated the desirability for determining the yields and properties of products isolated from the hulls at higher temperatures than of boiling water at atmospheric pressure. A series of experiments were made in which mucilages were extracted at 120°C. and 140°C. These temperatures were reached in a 4.5 liter autoclave under pressure. It was heated by direct flame. The blow valve in the cover was fitted with a pipe reaching within 1/8 inch from the bottom of the digester to allow removal of the liquid or dissolved contents by the internal steam pressure without removing the cover. Separation of the mucilage from the hull could then be made at 120°C. or any temperature above boiling water. The pipe was fitted with an 30 mesh sieve and the sieve surrounded with cloth towel- ing to allow filtering of the mucilage on blowing. For the 120°C. extract, 150 g. of crushed hulls were mixed with 1500 g. of tap water in the autoclave. The temperature was raised to 98°C. with the cover off and the contents continuously stirred to prevent local overheating. The cover was then fastened on and heating continued to 120°C. at which point it was held for 15 minutes (1/2 hour to temperature). The mucilage was then blown, slowly to allow time for filtration, into two 2-liter erlenmeyer flasks. It was carefully measured for yield. A second extraction was then made by forcing a liter of tap water into the digester through the blow valve without removing the cover, reheated to 120°C. and held there 15 minutes. The extract was again blown and collected in a separate flask for yield measurement and for individual tests of the two mucilages in paper. The solids content of each of the suspension was carefully measured and the total weight of solids removed calculated. The total yield was found to be 14.8% A.D. mucilage solids based on the original A.D. weight of hulls. The pH of the first extract was pH 5.6 and that of the second pH 5.3, both measured by the colorimetric method. These two extracts were applied to paper separately to determine any differences in their fiber deflocculating and adhesive properties.

Another series of extracts at high temperatures (140°C.) were made and applied as above. A similar procedure and the same apparatus was used as for the 120°C. extracts, with the following changes: (a) one hour was taken to reach the temperature, (b) it was blown as soon as the temperature reached 140°C. Four extractions were made and saved separately. They were blown slowly in each case to allow time for filtration. When the temperature reached 90°C. after blowing, about 980 g. of cold tap water were introduced

through the blow valve, the contents reheated to 140°C. (60 pound gauge pressure) and blown again. The color of these extracts was muddy brown. They were cloudy but stable. The pH of these four extracts varied between pH 4.5 and 4.7 despite the fact that alkaline tap water was used. This acid pH might be an indicator for the presence of uronic acids. Similar, although not strictly comparable cases are given in the literature where in a suspension or solution of plant material of any kind if the presence of uronic acids is suspected, it is subjected to prolonged dialysis so that all mineral salts and acids are removed, and then the undialyzable portion measured for its hydrogen ion concentration. If it is acid, the presence of uronic acids is definitely proven. Something similar might take place here when it is considered that most of the mineral acids and salts, if present, would have been removed in the first extraction, while the latter extracts still showing an acid reaction, might be acidic due to the difficulty soluble or suspendable uronic acid complexes. The solids removed in the mucilage from the 150 g. of hulls and the total yield, are shown in Table VI.

TABLE VI

140°C. water Extraction number	wt. suspension removed, g.	wt. air-dry solids removed in suspension, g.	Total air-dry yield cumulative
1st	754 g.	19.03 g.	12.7%
2nd	961	13.92	21.95
3rd	940	7.73	27.1
4th	923	3.73	29.6

[Extrapolating to 8 extractions - 31.8% yield possible]

As stated, the mucilage suspensions were collected separately and after the measurement of the solids in each, they were applied to paper pulp. These samples were included with those dry milled products prepared from the whole seed and dispersed as described. The test results are included in the same tables of data.

Conclusions:-

Cooking the dry milled bean gums before application to paper increased their effectiveness i.e., the fiber deflocculating and adhesive strength properties in paper were improved over those applied after cold water dispersing only (substantiating results obtained for imported locust bean gum some years ago). In addition it markedly increased the dispersibility of those dry milled samples on which the two methods were tried.

All of the samples applied gave positive results in paper but in

varying degrees. All showed reaction products with sodium tetraborate as evidenced from their wet strength imparting properties.

The honey locust bean gum samples prepared by General Mills, Inc., show the best all-around properties from a papermaking viewpoint. They were light in color, low in undispersible matter, and highly effective as fiber deflocculants and wet and dry strength improving agents for paper. Samples R21880 and R21876 (General Mills, Inc., numbers) were probably equal to the best grades of imported locust bean gum in all phases including color.

The mesquite bean gum and the palo verda bean gum (the General Mills' samples here tested) also showed good colors and good dispersibilities but were slightly inferior in deflocculating and wet and dry strength imparting properties in paper to the honey locust samples as well the dispersible portion of the flame tree seed mucilage, number R 21875.

The guar gum samples ("Rolled guar gum, alkaline") while slightly off color, were satisfactorily dispersible. They showed very good, but not the best, properties in paper from the standpoint of those here under discussion. Some improvements in milling conditions might possibly be found to brighten the color without interfering greatly with the other properties.

The dry milled "Cassia tora gum" and "Flame tree seed gum" contain too much inert material to be of any practical interest but that portion of the flame tree seed gum applied showed good properties except for color.

The soy bean hull mucilages are interesting not only because of their effectiveness along certain lines but because of the changes in their physical properties with changes in their methods of isolation. The concentrated 95°C. extract exhibited good adhesive properties, especially wet strength after borax treatment, but was not among the best as a fiber deflocculant. Extracted mucilages at 120°C. were more effective than those extracted with 140°C. water and repeated extractions at each temperature produced products of progressively weaker effectiveness and of lower yields. The optimum extraction temperature was not found i.e., optimum for yield and quality of the mucilage, but it has been demonstrated that this temperature lies somewhere between 95°C. and 140°C.

TABLE VII

## FORMATION NUMBERS (THWING) OF SHEETS TREATED WITH THE GUMS AND MUCILAGES

Gum or Mucilage Used, water dispersed portion passing 372.5 mesh wire used only. Per cent based on air-dry basis.	8% Addition	Rank of Effectiveness at 8% addition	4% Addition	Rank of Effectiveness at 4% addition	2% Addition	Rank of Effectiveness at 2% addition
Honey Locust Bean Gum, cooked General Mills, Inc., sample No. R21880, Alkali ground	43.1	1	43.3	3	43.6	2
Flame tree Seed Mucilage, cooked; Gen. Mills, Inc., sample No. R21875, Alkali ground	47.0	2	44.4	2	44.9	1
Honey Locust Bean Gum, cooked Gen. Mills, Inc., sample No. R21876, Alkali ground	44.9	3	47.6	1	41.2	5
Mesquite Bean Gum, cooked, Gen. Mills, Inc. Sample received 1/27/43	44.9	4	41.2	5	41.0	6
Soy Bean Hull Mucilage, 120°C. water extract, First extraction	44.7	5	42.7	4	43.0	3
Soy Bean Hull Mucilage, 120°C. water extract, Second extraction	43.7	6	39.7	9	42.6	4
Soy Bean Hull Mucilage, 95°C water extract, Evaporated to 8% solids at 60°C.	43.6	7	40.2	7	38.7	9
Palo Verde Gum, Uncooked Gen. Mills, Inc., sample No. 12/10/43	43.2	8	40.9	5	40.8	7
Soy Bean Hull Mucilage, 140°C. water extract, First extraction	42.6	9	40.1	8	39.6	8
Soy Bean Hull Mucilage, 140°C. water extract, Second extraction	40.0	10	37.7	10	37.9	10
Soy Bean Hull Mucilage, 140°C. water extract, Third extraction	39.5	11	37.4	11	34.4	12
Soy Bean Hull Mucilage, 140°C. water extract, Fourth extraction	38.4	12	36.3	12	35.9	11
Blank	27.9					

TABLE VII<sub>1</sub>

BURSTING STRENGTH OF SHEETS TREATED WITH  
THE GUMS, IN POUNDS PER SQUARE INCH

Gum or Mucilage Used, water dispersed portion passing 72.5 mesh wire used only. Per cent based on air- dry basis.	8% Addition	Rank of Effectiveness at 8% addition	4% Addition at 4% addition	Rank of Effectiveness at 4% addition	2% Addition at 2% addition	Rank of Effectiveness at 2% addition
Honey Locust Bean Gum, Cooked, Gen. Mills, Inc. Sample No. R21880 Alkali Ground.	39.1	1	35.5	4	34.1	1
Soy Bean Hull Mucilage, 95°C. Water extract, Evaporated to 8% solids at 60°C.	38.7	2	35.9	3	31.1	4
Honey Locust Bean Gum, Cooked, Gen. Mills, Inc. Sample No. R21876 Alkali Ground.	38.6	3	36.1	2	31.6	3
Palo Verde Gum, Uncooked, Gen. Mills, Inc., Sample of 12/10/42.	37.9	4	33.1	5	29.1	6
Flame Tree Seed Mucilage, cooked, Gen. Mills, Inc. Sample No. R21875 Alkali Ground.	34.5	5	36.2	1	32.1	2
Mesquite Bean Gum, Cooked, Gen. Mills, Inc., Sample received 1/27/43.	33.5	6	32.1	6	29.2	5
Soy Bean Hull Mucilage, 140°C. water extract, First extraction.	32.5	7	31.3	8	28.8	7
Soy Bean Hull Mucilage, 120°C. water extract, Second extraction.	31.5	8	29.1	9	27.9	8
Soy Bean Hull Mucilage, 120°C. water extract, Third extraction.	31.4	9	31.9	7	27.0	10
Soy Bean Hull Mucilage, 140°C. water extract, Second extraction.	29.4	10	28.6	10	27.2	9
Soy Bean Hull Mucilage, 140°C. water extract, Third extraction.	26.9	11	25.5	11	23.5	12
Soy Bean Hull Mucilage, 140°C. water extract, Fourth extraction.	24.2	12	24.5	12	24.4	11
Blank	20.4					

TABLE IX

WET STRENGTHS OF SHEETS TREATED WITH THE  
GUMS AND BORAX, IN WET TENSILE UNITS\*

Gum or Mucilage used, Water dispersed portion passing 72.5 mesh wire used only. Per cent based on air- dry basis.	Rank of Effectiveness Addition at 8% addition	Rank of Effectiveness Addition at 4% addition	Rank of Effectiveness Addition at 2% addition			
Soy Bean Hull Mucilage, 95°C. water extract, Evaporated to 8% solids at 60°C.	12.7	1	10.7	1	7.5	5
Honey Locust Bean Gum, cooked, Gen. Mills, Inc., sample No. R21880, Alkali Ground	11.9	2	9.7	5	8.9	1
Honey Locust Bean Gum, cooked, Gen. Mills, Inc., sample No. R21876, Alkali Ground	11.6	3	10.5	2	8.8	2
Mesquite Bean Gum, cooked, Gen. Mills, Inc., sample received 1/27/43	11.0	4	9.8	4	8.6	4
Flame Tree Seed Mucilage, cooked, Gen. Mills, Inc. Sample No. R21875, Alkali ground	10.4	5	10.3	3	8.8	3
Palo Verde Gum, uncooked, Gen. Mills, Inc., Sample of 12/10/42	8.8	6	9.1	6	7.0	6
Soy Bean Hull Mucilage, 120°C. water extract, First extraction	8.8	7	7.5	7	6.4	7
Soy Bean Hull Mucilage, 140°C. water extract, first extraction	7.8	8	7.2	8	6.1	8
Soy Bean Hull Mucilage, 120°C. water extract, Second extraction	7.7	9	6.7	9	5.5	9
Soy Bean Hull Mucilage, 140°C. water extract, Second extraction	7.7	10	5.6	10	5.3	10
Soy Bean Hull Mucilage, 140°C. water extract, Third extraction	4.2	11	3.3	11	2.8	11
Soy Bean Hull Mucilage, 140°C. water extract, Fourth extraction	3.3	12	3.2	12	2.7	12
Blank	2.3					

\*Gum treated sheets given 1% borax dippings and redried. Strip 15 mm. wide tested.

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Mr. Steele

Mr. Rowland

Mr. Frommuller

PROJECT NO. 849

COOPERATOR Institute

REPORT NO. 1

DATE Feb. 12, 1943

NOTE BOOK 416

PAGE 6 TO 59

SIGNED

D. Frommuller

## BY-PRODUCTS DERIVED FROM THE GUAR AND HONEY LOCUST EMBRYOS

### Work Done.

A series of preliminary experiments were carried out in order to isolate the more important by-products from guar and honey locust embryos. It has been shown in previous reports that the oils isolated from these sources are at the present time considered to be of little value. This is based primarily upon the low oil content and the cost of extracting this oil. The oils would be of great economic importance if the sterols were of exceptional potency as vitamins. However, the latest advice from General Mills is that the sterols are of little value because of low yields. It was noted that the Vitamin E concentration was approximately 100% greater than that obtained from wheat germ oil.

The proteins were investigated next as a possible by-product since the oils showed little promise of being produced economically. The proteins were studied for the primary purpose of investigating their use as a coating color adhesive.

The proteins were isolated by several methods. The proteins as isolated appear to be largely albuminoids mixed with glutelins and prolamines. The mixture was separated and the albuminoids and glutelins used as coating adhesives. Experiments with these compounds and with the original mixture of protein indicate that they are poor coating adhesives because as isolated they tended to produce extremely fine stable foam where ball milled in the presence of clay. During the separation of the protein it was noted during certain steps that considerable amounts of foam were produced. It was thought that a foreign substance similar to saponin might be largely responsible for the foam. Attempts were made to isolate this substance which have proved unsuccessful to the extent that a material has been isolated which partially accounts for the foam, but the proteins themselves show the tendency to form very stable foams. For this reason the protein as a coating adhesive must be discarded. A 5% dispersion of the protein when beaten for 5 minutes with a mixmaster gave a foam similar in nature to that obtained with egg white. The indications are that this material should be investigated further as a substitute for egg albumin.

Incidental to the isolation of the proteinaceous compounds from guar was the separation of a purple pigment. This pigment as isolated does not appear to be a chloroplast, and at the present time the nature of this material is unknown.

#### Experimental Procedure.

##### GUAR

##### (a) Isolation of the guar pigment.

Approximately 50 g. of whole guar beans were placed in a separatory funnel and wet steam injected into the mass. The stopcock was kept open during the steaming so that the condensate was removed as rapidly as it collected in the bottom of the funnel. The beans were steamed for one-half hour. At the end of this time the condensate was practically clear. The condensate was filtered and filtrate evaporated to dryness in vacuo. A purple crystalline product was obtained. The following tests were made in an attempt to classify the material.

1. Effect of dilute acetic acid - color immediately discharged.
2. Effect of dilute sodium hydroxide - color changes from purple to a deep wine red.
3. Acidulated amyl alcohol - color immediately discharged. No evidence of being soluble in alcohol.
4. Zn dust and hydrochloric acid - color discharged. Was not reoxidized on standing.
5. Acidified solution so as not to discharge color and heated for 30 min. on a steam bath, cooled and added tertiary amyl alcohol. The color was soluble in the alcohol layer.
6. The substance was insoluble in: 50% ethyl alcohol, acetone, 85% acetone, propylene, glycol, ethylene glycol, cello-solve, methyl cellosolve, ether, chloroform, carbon tetrachloride, dioxane, pyridine, methyl amyl ketone, cyclohexanone, monochlor benzene, furfural, ethyl acetate, octyl acetate, xylene.

The fact that it is insoluble in 85% acetone indicates that it is not a chloroplast. The only solvent found was water. The aqueous solution is purple with a pH of 4.5.

(b) Isolation of Protein.

Several preliminary experiments were made in order to determine the best means of isolating the protein from the ground seeds and from the ground embryos. It was found early in the investigation that the proteins could only be isolated from the ground seed with great difficulty. Therefore, the embryos were isolated from the endosperms and seed coats by a flotation method using a mixture of acetone and chloroform as the vehicle. However, before the proteins could be isolated from the embryos a three-pound sample of embryo flour had been received from General Mills. The embryo separated by milling techniques was used in the experiments described below in preference to the flour prepared by the flotation method. This was done primarily to save time since the milled product had been previously extracted with petroleum ether.

One hundred g. of guar embryo flour were suspended in 1500 g. of water at 77-82° C. and 10 ml. of 10% sulfuric acid were added to reduce the pH of 4.7. An outside indicator (chlorophenol red) was used to indicate changes in pH. The suspension of the flour was stirred for 2 hours at a constant temperature and pH, 1 ml. of 10% sulfuric being added after 1 hour. At the end of two hours the suspension was allowed to settle and the dark yellowish brown supernatant liquor was decanted. Three hundred ml. of liquor was removed by decantation and 300 ml. acidulated water, pH of 4.7, was added. The suspension was then allowed to stand for 1 1/2 hours. Six hundred fifty ml. of supernatant liquor was removed by decantation and 650 ml. of distilled water was added at 70° C. The temperature of the suspension was 42° C. and this temperature was maintained throughout the rest of the procedure.

Three g. of NaOH was dissolved in 30 ml. of distilled water and added to the suspension, and suspension was stirred for 30 min.; 3.4 g. of CaO was suspended in 30 ml. of distilled water and added to the suspension. The suspension was stirred for 1 1/2 hours. The color changed from a light cream color to a greenish yellow color. The resulting suspension was centrifuged in the Sharples centrifuge. The residue was washed three times and suspension centrifuged between each washing. The washings were combined with the original centrifugate and the dispersion was placed in a constant temperature bath at 42° C. for 18 hours. The dispersion was removed from the bath and acidified with 21.7 ml. of 10% sulfuric acid to a pH of 4.8. Precipitate settled rapidly and

and the supernatant liquor was removed by decantation. The precipitate was washed twice with distilled water at pH of 4.8 and then filtered. The residue was suspended in acetone, filtered and air dried. Yield of air dried protein was 8 g. (41-G). It had a dark cream color and was granular in appearance. It also appeared to be more hydrous than casein or soya protein.

The low yield was not in accord with the nitrogen analysis which indicated that 51.8% of the flour was protein (v.f. 6.25). A portion of this material was used to make a coating color. This was discarded because of foam and poor pick and spreading qualities. Therefore, a series of experiments were made as follows:

Forty g. of flour were suspended in 500 g. of 10% NaCl solution, warmed to 60° C. and maintained at this temperature for one-half hour. The suspension was centrifuged, and residue washed with 10% NaCl at 60° C., centrifuge. Distilled water at 60° C. was added to the combined centrifugates until the dispersion was turbid. The suspension was heated on a steam bath for one-half hour and then cooled to 25° C. The suspension was allowed to stand two days and then filtered, and residue washed and dried by means of acetone. Two g. of residue labeled 43-1 was a dark cream color solid.

The filtrate was saturated with  $(\text{NH}_4)_2\text{SO}_4$  and a flocculent tan precipitate was thrown out of solution. The precipitate was removed by filtration and filtrate evaporated. The residue was dispersed in distilled water and dialysed for 50 hours; a small amount of toluene was added as a preservative. The precipitate was removed by filtration and dried with acetone. Dry material to the amount of 6.5 g. were obtained. This product was labeled 44-2. It was a light cream color. The filtrate upon addition of acetone flocced, floc filtered and dried. Yield was 2.0 g. and labeled 44-3. This product was white.

The residue from the initial salt extraction was suspended in 85% alcohol for 48 hours. The alcohol was removed by filtration and the alcohol evaporated on a steam bath to dryness. Seven g. of a yellowish brown product was obtained.

The residue after the alcohol extraction weighted 11 g. This material was dispersed in 150 g. of water. A thick viscous suspension resulted which did not thin out upon the addition of 3.0 ml. of 10% NaOH, and raising the temperature to 45° C. 100 g. of water and 34 g. of CaO were added after one-half hour. The suspension was heated for 1 1/2 hours at 45° C. The suspension was

centrifuged and centrifugate allowed to stand 2 1/4 hours before acidifying. The dispersion was acidified with 10% H<sub>2</sub>SO<sub>4</sub> and allowed to stand overnight. The precipitate was removed by filtration and the residue dispersed in acetone, filtered and dried. A white solid to the amount of 1.5 g. was obtained. The yields and per cent nitrogen are presented in Table I. A schematic diagram of the methods of separation are presented in Figure 1.

TABLE I  
YIELD AND NITROGEN CONTENT OF VARIOUS PROTEIN COMPONENTS IN  
GUAR EMBRYO FLOUR

Sample	-----Yield-----			
	g.	% Flour	% Theoretical Protein in Flour*	% Nitrogen
43-1 Albuminoids & Globulins	2.0	5.00	9.70	10.61
44-2 Globulins (dialysis)	6.0	15.00	29.10	17.74
44-3 Globulins (acetone)	1.5	3.75	7.28	—
45-1 Glutelins	1.5	3.75	7.28	14.39
44-1 Prolamines	7.0	17.50	33.98	1.49
Guar Embryo Flour	—	—	—	8.28
Guar Embryo Flour ext. with CH <sub>3</sub> OH	—	—	—	12.38
Total	18.0	45.0	90.34	

\*This value is based upon 8.28% nitrogen in the flour and protein content =  $8.28 \times 6.25 = 51.8\%$

The salt soluble and alkali soluble products when tested for foam qualities indicated that the tendency to foam when dispersed was markedly decreased. However, it was decided to find out if a natural saponin was present in the flour and if so the most effective way to isolate it.

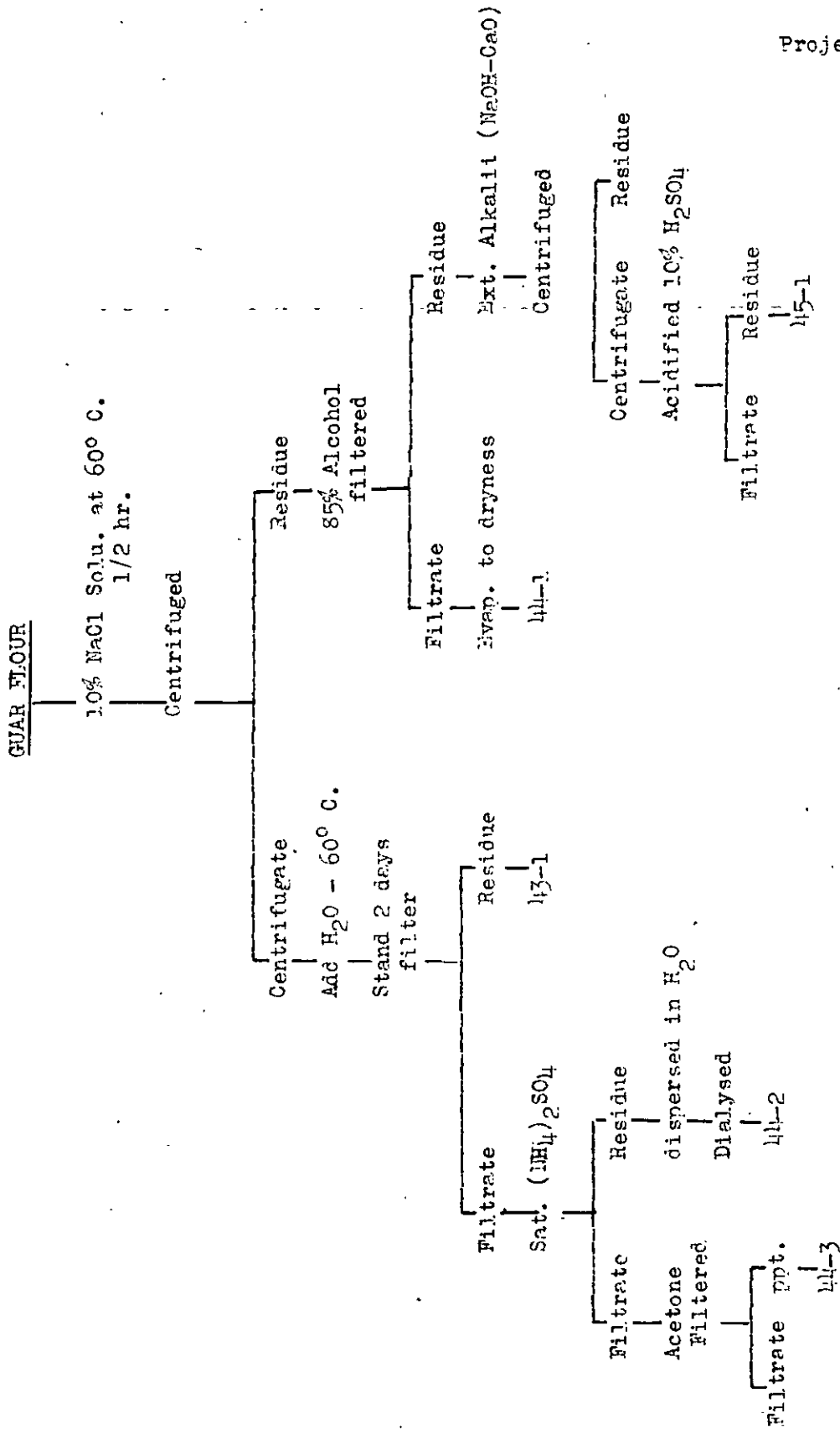


FIGURE 1.

As a means of isolating the saponin or foam producing material, an experiment was carried out using the following procedure:

Fifty g. of guar embryo flour, which has previously been extracted with petroleum ether, were extracted with 200 ml. of boiling 80% alcohol for 15 min. The extract removed by filtration and residue was extracted twice more. The alcoholic extract had an orange brown color. Upon cooling a yellow precipitate formed. This precipitate was filtered and recrystallized from 80% boiling alcohol, and dried with acetone. A wax-like product was obtained which on dispersing in water showed no tendency to foam. (46-1)

The alcoholic extracted residue was extracted with 750 ml. of acidified distilled water for 2 hours at 60° C. The pH of the extraction media was 4.8. The extract was allowed to stand one-half hour and filtered. The filtrate had a light straw color and was evaporated to dryness after neutralizing with 10% NaOH. As soon as the temperature reached 80° C. a floc formed. The dry residue after evaporation was extracted with four 50 ml. portions of boiling ethyl alcohol. Upon evaporation to 50 ml. and cooling a flocculent brown precipitate formed. The extract was centrifuged and precipitate dried over acetone. This product was readily soluble in boiling water. The material was dispersed in hot water, cooled, and filtered. The filtrate, straw-yellow in color, was evaporated to dryness. (47-2). The residue was a white amorphous solid. (46-2). Alcoholic centrifugate was evaporated to dryness. (47-1)

The products obtained by the above treatments are listed below:

- 46-1 A straw-colored wax-like material - less than 0.5g.
- 46-2 A white amorphous solid - soluble in dilute acids, insoluble in water. Millons test negative.
- 47-1 A grayish brown solid - soluble in water and alcohol, shows a tendency to foam. Negative test for proteins.
- 47-2 A brown brittle solid - readily soluble in water. Positive Biuret test.
- 47-3 Alcohol soluble fraction from original extraction which did not crystallize on standing. Upon evaporation a brown sticky substance was obtained, soluble in hot water. Negative Biuret and Millons test.

The protein grits from the alcoholic and acid extractions were extracted with alkalis as previously described on p. 3. Eleven g. of an amorphous cream-colored solid were obtained. (49-1) Positive Millon, Biuret, Rosenheim, Molisch, p-dimethyl aminobenzaldehyde tests were obtained.

Although the yield had increased from 8% to 22% of alkali soluble protein, the tendency to foam was still pronounced. Another experiment was carried out repeating the procedure outlined on p. 3 except that instead of centrifuging the alkaline extract immediately after 2 hours, the suspension was allowed to stand overnight. The products obtained and yields on an as is basis are listed below.

50-1	Acid soluble fraction	41.0 g.
50-3	Alkali soluble fraction	33.0
51-1	Alkali soluble fraction ppt. acetone.	7.3
51-2	More precipitable with acetone	16.0
50-2	Residue	12.0
		<u>109.3</u> g. recovered

Fraction 51-2 gave positive Biuret and Ninhydrin tests.

If fractions 50-3 and 51-1 are combined, the yield as alkali derived protein is 40.3%. This is the best yield obtained thus far. A coating color was prepared using fraction 50-3. Protein to the amount of 11.3 g. was dispersed in 60 g. of water containing 1.13 g.  $\text{Na}_2\text{CO}_3$  at 50° C. for 20 min. Eighty-six g. of water and 2 drops sodium silicate were added to 87 g. of English coating clay and mixed in a mortar and pestle. The clay slurry was placed in a ball mill and the protein dispersion added. After milling for 5 min., the mill was opened and the coating color was a mass of stiff solid foam. Four drops of capryl alcohol were added and milling continued for 25 min. The color was a mass of stiff foam and as such could not be used. The foam did not break upon the further addition of capryl alcohol.

A portion of fraction 50-3 was extracted with boiling 80% methanol for 5 min. The extracted protein freed of alcohol, when dispersed in alkali showed less tendency to foam than the unextracted. Upon the basis of this observation another attempt was made to use boiling alcohol in the hope that prolonged heating might remove the compounds responsible for the foam and at the same time insolubilize the heat coagulable proteins.

One hundred g. of guar flour were refluxed twice with 540 ml. of 80% methanol. The flour separated by filtration and filtrates kept separate. The first filtrate on cooling contained a fine white precipitate, which was separated by centrifuging. The material was redissolved in 80% boiling alcohol. The second filtrate or extract changed from a light straw color to a reddish brown color upon standing.

The alcohol extracted grits were then treated as described on p. 3. Eleven g. of a cream colored solid were obtained which was labeled 54-1. Dispersions of this material when heated showed a tendency to foam and to a more marked degree than any of the proteins isolated.

It was thought the substances responsible for the foam might be water soluble or that the protein was similar in character to albumins which show a tendency to foam. One hundred g. of guar embryo flour were suspended in 1500 ml. of distilled water and the pH adjusted to 7.3 with 3 drops of 10% NaOH. The suspension was stirred for 2 hours at 25° C., settled and the supernatant liquor removed by decantation. The extractions were repeated until 6000 ml. of extract were obtained. The aqueous extracts were acidified to pH 4.8 with 10% H<sub>2</sub>SO<sub>4</sub>. The precipitate was removed by means of a centrifuge and washed with water at pH 4.3 until foam no longer formed when the wash water was violently agitated. The residue was dried with acetone. (56-1) The wash liquor was combined with the centrifugate and evaporated to dryness on a steam bath.

The residue from the original extract appeared to be very small. Therefore, the above experiment was repeated, except that aqueous extracts were allowed to stand overnight. The extracts were preserved with toluene. The precipitate which formed was removed by centrifuging and the precipitate was washed 3 times with water at pH 4.5. The product was dried with acetone. A very light cream colored material to the amount of 30.6 g. was obtained. (57-1) The extracted grits were then extracted with alkali as previously described and 12.3 g. (57-2) of alkali derived protein were obtained.

Five g. of fraction 57-1 were dispersed in 95 g. water and the dispersion beaten in a Mixmaster for 5 minutes at No. 8 speed setting. A stable fine compact bubble foam resulted which did not rupture on standing. A portion of the foam was dried in the oven and showed a tendency to collapse on heating.

A series of qualitative protein tests were made on samples 57-1 and 57-2. The results are as follows:

Test	57-1	57-2
Biuret	Reddish pink	Bluish pink
Millons	Positive	Negative
Liebermans	Black	Red-purple
Rosenheim	Purple	Yellow
Molisch	Positive	Positive
Adamkilwicz	Positive	Negative
Ninhydrin	Negative	Negative
p-Dimethylaminobenzaldehyde	Strongly positive	Positive

The indications as a result of these tests are that Protein 57-1 there is the presence of a peptide linkage, hydroxy benzene nucleus, a carbohydrate group in the protein molecule, and the presence of tryptophane or other indole derivatives. Whereas protein 57-2 differs from protein 57-1 in that the hydroxy benzene nucleus is absent and that all of the tests for tryptophane or indole derivatives are not consistent. This may indicate that a small percentage of tryptophane is present in sample 57-2.

#### HONEY LOCUST

The protein was isolated from a petroleum ether extracted flour from the embryos of the honey locust seed. The method employed was similar to that previously described on p. 3 for the isolation of the guar protein. Forty-nine g. of alkali derived protein were obtained from 100 g. of flour. The tendency to foam was not as marked and where used in a coating color the foam which formed was easily dissipated upon the addition of two drops of capryl alcohol. The adhesive qualities of this protein were on a par with casein. The protein was qualitatively tested as outlined on p. 10 above. Positive Biuret, Millons, Rosenheim, Molisch, weakly positive p-dimethylaminobenzaldehyde tests, and negative Ninhydrin, Liebermans, and Adamkilwicz tests were obtained with this material. These qualitative tests indicate that this protein is similar in character to that obtained with the guar fraction 57-2. However, from the yields it would appear that there is at least 5-6 times as much alkali derived protein in the honey locust embryo flour as compared to the guar embryo flour.

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COOPERATOR Institute  
REPORT NO. 9  
DATE July 19, 1943 (Typed 7/22/43)  
NOTE BOOK 223  
PAGE 66713 TO 66720  
SIGNED N. A. Kjelson

N. A. Kjelson

## Subject:

Critical evaluation of the dry-milled gum samples by G.M.I. of guar, honey locust, and flame tree. G.M.I. numbers R 22539, R 22525 and R 22470.

## Procedures and Results:

The application procedures were similar to those previously described. The tables I to IX accompanying, are self-explanatory. A summary of the results is given in Table IX.

TABLE I

## BRIGHTNESS OF SHEETS CONTAINING THE DRY-MILLED GUMS, UNAGED SHEETS

Gum Used	% Decrease from			% Decrease from			% Decrease from		
	8% Addition*	Unaged blank	Rank at 8%	2% Addition*	Unaged blank	Rank at 2%	1% Addition*	Unaged blank	Rank at 1%
Locust bean gum, Imported, uncooked	78.7	1.13	1	79.2	0.502	2	79.1	0.628	4
Locust bean gum, Imported, cooked	78.5	1.38	2	78.5	1.38	3	78.8	1.00	5
G-11 Honey Locust (May 1943, Gen. Mills Sple # R22525) cooked	78.2	1.76	3	78.4	1.51	4	79.3	0.376	1
G-11 Honey Locust (May 1943 GMI sample #R22525) Uncooked.	78.0	2.01	4	79.9	-	1	79.3	0.376	3
G-4-2 (June 1943 GMI sample # R22539) Guar, uncooked.	75.4	5.28	5	78.0	2.01	5	79.3	0.376	2
G-4-2 Guar (June 1943 GMI sample # R22539) cooked.	73.4	7.79	6	77.1	3.14	6	78.1	1.88	6
G-9 Flame Tree (June 1943 GMI sample #R22470) uncooked.	64.9	18.47	7	73.4	7.79	7	76.3	4.15	7
G-9 Flame Tree (June 1943 GMI sample #R22470) uncooked.	63.4	20.35	8	72.3	9.17	8	75.2	5.53	8
Blank (unaged)	79.6								

\* General Electric Reflection Meter Brightness. Method: TAPPI T217 sm-41.

TABLE II

BRIGHTNESS OF SHEETS CONTAINING THE DRY-MILLED GUMS, SHEETS OVEN AGED 72 HOURS AT 105° C.

Gum Used	% Decrease Rank			% Decrease Rank			% Decrease Rank		
	8% Addition*	from Aged blank	at 8% Addition	2% Addition*	from Aged blank	at 2% Addition	1% Addition*	from Aged blank	at 1% Addition
G-11 Honey Locust (GMI sample # R22525), cooked.	57.2	1.38	1	58.5	-	1	58.6	-	1
Locust Bean Gum, Imported, uncooked.	56.9	1.90	2	57.7	0.517	5	56.9	1.90	7
G-11 Honey Locust (GMI sample #R22525), uncooked.	56.8	2.07	3	57.6	0.689	6	58.0	-	4
G4-2 Guar (GMI sample #R22539), uncooked.	56.0	3.45	4	58.0	-	3	57.8	0.344	5
G4-2 Guar (GMI sample #R22539), cooked.	55.8	3.79	5	57.9	0.172	4	58.1	-	3
Locust Bean Gum, Imported, cooked.	55.5	4.31	6	58.0	-	2	57.6	0.689	6
G-9 Flame Tree (GMI sample #R22470), uncooked	51.5	11.21	7	57.0	1.72	7	58.2	-	2
G-9 Flame Tree (GMI sample #R22470), cooked.	50.2	13.45	8	55.8	3.79	8	56.4	2.76	8
Blank (aged)	58.0								

\* General Electric Reflection Meter Brightness. Method: TAPPI T217 sm-41.

TABLE III

## FOLDING ENDURANCE OF SHEETS CONTAINING THE DRY-MILLED GUMS. SHEETS UNAGED.

Gum Used	8% Addition*	Fold % Increase Over blank	Relative Rank at 8%	4% Addition*	Fold % Inc. Over blank	Relative Rank at 4%	2% Addition*	Fold % Increase Over blank	Relative Rank at 2%
Locust Bean Gum, Imported, uncooked.	252	2700.0	1	137	1422.2	1	96	966.7	1
G-11 Honey Locust (GMI sample #R22525) Cold water 17 hr. and cooked.	202	2144.4	2	123	1266.7	2	60	566.7	6
G-9 Flame Tree (GMI sample # R22470), uncooked.	198	2100.0	3	104	1055.6	5	63	600.0	5
G-11 Honey Locust (GMI sample #R22525), uncooked.	196	2077.8	4	118	1211.1	3	75	733.3	2
Locust Bean Gum, Imported, cooked.	151	1577.8	5	114	1166.7	4	68	655.6	3
G-11 Honey Locust (GMI sample #R22525), cooked.	122	1255.6	6	95	955.6	6	58	544.4	7
G-9 Flame Tree (GMI sample #R22470), cooked.	106	1077.8	7	63	600.0	7	65	622.2	4
G4-2 Guar (GMI sample #R22539), uncooked.	80	788.8	8	50	455.5	9	41	355.6	9
G4-2 Guar (GMI sample #R22539), cooked.	70	677.8	9	54	500.0	8	45	400.0	8
Blank (1)	10								
Blank (2)	8								
Average	9								

\* M. I. T. Double folds.

TABLE IV

FOLDING ENDURANCE OF SHEETS CONTAINING THE DRY-MILLED GUMS.  
SHEETS OVEN AGED 72 HOUR AT 105° C.

Gum used	8% Addition*	Fold %			4% Addition*	Fold %			2% Addition*	Fold %		
		Inc. over Aged Blanks	Relative Rank at 8%	Fold % Decrease on Aging**		Inc. over Aged Blanks	Relative Rank at 4%	Fold % Decrease on Aging**		Inc. over Aged Blanks	Relative Rank at 2%	Fold % Decrease on Aging**
G-11 Honey Locust (GMI sample #R22525) Cold water 17 hr. and cooked.	55	400.0	1	72.77	34	209.1	4	72.36	35	218.2	1	41.67
Locust bean gum, Imported, uncooked.	51	363.6	2	79.76	39	254.5	3	71.53	27	145.4	4	71.88
G-11 Honey Locust (GMI sample #R22525) Uncooked.	47	327.3	3	76.02	40	263.6	1	66.10	31	181.8	3	58.67
G-11 Honey Locust (GMI sample #R22525), cooked.	43	290.9	4	64.75	40	263.6	2	57.89	26	136.3	5	55.17
G-9 Flame Tree (GMI sample #R22470) cooked	39	254.5	5	63.21	34	209.1	5	46.03	34	209.1	2	47.69
Locust Bean gum, Imported, cooked.	35	218.2	6	76.82	30	172.7	7	73.68	26	136.3	6	61.76
G-9 Flame Tree (GMI sample #R22470) uncooked.	34	209.1	7	82.83	33	200.0	6	68.27	25	127.3	7	60.32
G4-2 Guar (GMI sample #R22539), cooked.	33	200.0	8	52.86	27	145.4	9	50.00	24	118.2	8	46.67
G4 G4-2 Guar (GMI sample #R22539), uncooked.	27	145.4	9	66.25	29	163.6	8	42.00	23	109.1	9	43.90
Blank (Average)	11											

\* M. I. T. Double Folds.

\*\* % Decrease from unaged samples corresponding. See Table III.

TABLE V

## THWING FORMATION NUMBERS OF SHEETS CONTAINING THE DRY-MILLED GUMS.

Gum Used	% Increase Rank at 8%			% Increase Rank at 4%			% Increase Rank at 2%		
	8% Addition*	Blank	Relative Rank at 8% Addition	4% Addition*	Blank	Relative Rank at 4% Addition	2% Addition*	Blank	Relative Rank at 2% Addition
G-11 Honey Locust (GMI sample #R22525), Cooked.	45.8	70.26	1	40.6	48.70	6	39.6	47.21	5
Locust Bean gum, Imported, cooked.	44.3	64.68	2	42.4	57.62	4	42.8	59.11	1
G-11 Honey Locust (GMI sample #R22525), Cold water 17 hr. and cooked.	43.1	60.22	3	43.5	61.71	1	41.1	52.79	2
Locust Bean gum, Imported, uncooked.	41.7	55.02	4	42.8	59.11	3	37.6	39.78	7
G-11 Honey Locust (GMI sample #R22525), Uncooked.	41.3	53.53	5	43.0	59.85	2	40.8	51.67	3
G-9 Flame Tree (GMI sample #R22470), cooked.	39.4	46.47	6	41.7	55.02	5	40.1	49.07	4
G4-2 Guar (GMI sample #R22539), cooked.	38.5	43.12	7	39.7	47.58	7	32.9	22.30	9
G-9 Flame Tree (GMI sample #R22470), Uncooked.	38.0	41.26	8	37.7	40.15	8	37.9	40.89	6
G4-2 Guar (GMI sample # R22539), uncooked.	35.7	32.71	9	35.0	30.11	9	35.5	31.97	8
Blank (1)	27.2								
Blank (2)	26.6	Average	Blank	26.9					

\* Thwing Formation Number.

TABLE VI

## BURSTING STRENGTHS OF SHEETS CONTAINING THE DRY-MILLED GUMS.

Gum Used	% Relative Increase Rank at 8%			% Relative Increase Rank at 4%			% Relative Increase Rank at 2%		
	8% Addition*	Blank	at 8% Addition	4% Addition*	Blank	at 4% Addition	2% Addition*	Blank	at 2% Addition
Locust Bean gum, Imported, uncooked.	39.8	117.5	1	35.7	95.08	2	34.4	87.98	1
G-11 Honey Locust (Gen. Mills, Inc. sample #R22525), Cold water 17 hr. and cooked.	39.1	113.7	2	36.2	97.81	1	32.9	79.78	3
G-11 Honey Locust (GMI sample #R22525), uncooked.	38.4	109.8	3	34.6	89.07	4	33.1	80.87	2
G-11 Honey Locust (GMI sample #R22525), cooked.	38.1	108.2	4	34.3	87.43	5	32.2	75.96	5
G-9 Flame Tree (GMI sample #R22470), uncooked.	37.1	102.7	5	34.2	86.89	6	30.7	67.76	7
Locust Bean gum, Imported, cooked.	36.2	97.81	6	34.9	90.71	3	31.3	71.04	6
G-9 Flame Tree (GMI sample #R22470), cooked	34.8	90.16	7	32.4	77.05	7	32.3	76.50	4
G4-2 Guar (GMI sample #R22539), uncooked.	34.0	85.79	8	29.6	61.75	9	28.3	54.64	8
G4-2 Guar (GMI sample #R22539), Cooked.	32.4	77.05	9	31.3	71.04	8	28.0	53.01	9
Blank (1)	18.4								
Blank (2)	18.2								
	Blank Average		18.3						

\* Bursting Strength Points.

TABLE VII

## WET STRENGTHS OF SHEETS CONTAINING THE DRY-MILLED GUMS.

Gum Used	8% Increase over Blank			4% Increase over Blank			2% Increase over Blank		
	8% Addition*	% over Blank	Relative Rank at 8%	4% Addition*	% over Blank	Relative Rank at 4%	2% Addition*	% over Blank	Relative Rank at 2%
Locust Bean Gum, Imported, Uncooked.	13.9	768.8	1	12.1	656.3	4	10.2	537.5	5
G-11 Honey Locust (GMI sample #R22525), Cold water 17 hr. and cooked.	13.1	718.8	2	12.5	681.3	1	10.9	581.3	4
G-11 Honey Locust (GMI sample #R22525), uncooked.	13.0	712.5	3	12.5	681.3	2	11.7	631.3	1
G-11 Honey Locust (GMI sample #R22525), cooked.	12.9	706.3	4	11.7	631.3	5	11.2	600.0	2
Locust Bean Gum, Imported, cooked.	12.6	687.5	5	12.2	662.5	3	11.2	600.0	3
G-9 Flame Tree (GMI sample #R22470), uncooked.	12.5	681.3	6	10.9	581.3	6	9.8	512.5	6
G-9 Flame Tree (GMI sample #R22470), cooked.	11.9	643.8	7	10.9	581.3	7	9.8	512.5	7
G4-2 Guar (GMI sample #R22539), cooked.	11.2	600.0	8	9.2	475.0	8	8.0	400.0	8
G4-2 Guar (GMI sample #R22539), uncooked.	10.2	537.5	9	8.1	406.3	9	7.5	368.8	9
Blank (1)	1.6								
Blank (2)	1.5								
Blank Average	1.6								

\* Units per 15 mm. strip.

TABLE VIII

OSTWALD PIPETTE VISCOSITIES AT 30° C. OF DRY-MILLED GUMS COOKED TO 95° C. RELATIVE VISCOSITY\*.

Gum Used	0.10% Solids	0.20%	0.30%	0.40%	0.50%
G-11 Honey Locust (Gen.Mills Inc. sample #R22525).	2.210	6.057	16.838	45.810	116.267
Locust Bean Gum, Imported.	2.152	5.981	16.324	43.771	101.676
G-4 Guar (GMI sample #R22376).	1.790	3.638	7.695	17.200	37.486
G-4-2 Guar (GMI sample #R22539).	1.352	1.829	2.724	4.019	6.038
G-9 Flame Tree (GMI sample #R22470).	1.161	1.352	1.600	1.924	2.305

\* Pipette flow time for water only, 5.25 seconds.

TABLE IX

## CAPITULATION OF PROPERTIES OF THE DRY-MILLED GUMS FOR PAPERMAKING PURPOSES.

Gum Used	Color	Odor	Vis- cosity	Dispersi- bility	Foaming Tendency	Fiber Deflocc- ulant	Bursting Strength Properties	Papermaking Properties		General Acceptability of Paper purposes (Estimate)
								Wet Strength with Borax Properties	Folding Endurance Properties	
G-9 Flame Tree (GMI) #R22470).	Poor	V. Sl.	V. Low	Good	None	Poor	Fair	Fair	Fair	Poor
G-2 Guar (GMI, #R22539).	Fair- Poor	Strong	Low	Good	Some	Poor	Poor	Fair	Poor	Poor
G-4 Guar (GMI, #R22376).	Fair- Good*	Slight	Mod. High	Fair	None	Good**	-	Good**	-	Fair-good
G-11 Honey Locust (GMI #R22525).	Good	None	High	V. Good	None	Good	Good	Good	Good	Good
Imported Locust Bean Gum.	Good	V. Sl.	High	Mod. Good	None	Good	Good	Good	Good	Good

\* When at papermaking acidity range.

\*\* Not determined but estimated from other properties.

# PROJECT REPORT FORM

PROJECT NO. 849 X

COOPERATOR Institute

REPORT NO.

DATE June 22, 1943

NOTE-BOOK 503

PAGE 5 TO 36

SIGNED Ernest Anderson

Ernest Anderson

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Rowland  
Fronmuller  
Anderson

## Report on the Composition of the Endosperm Mucilages

### From Seeds of Various Legumes.

This report will deal with the qualitative composition of the mucilages, i.e. what substances are present in them. A later report will deal with the quantitative composition of these materials.

#### The Literature

Early work on carob mucilage and the mucilages from lucerne and fenugreek is reviewed in Wiesner, die Rohstoffe des Pflanzenreiches, Vol. 2 pp. 1867-1869. Some of the earlier workers reported the presence of glucose and fructose as well as mannose and galactose among the products of hydrolysis of these mucilages. Bourquelot and Heressey reported that the mucilages from carob seed, fenugreek and alfalfa seed were manno-galactons. Daoud, [Biochem. J. 26, 255 (1932)] made a very careful study of the mucilage from fenugreek. He concluded that this mucilage is a manno-galacton and that it contains no d-glucose. May and Schulze, [Z. Biol. Chem. 97, 201, (1936)] have also reported some work on the mucilage from lucerne (alfalfa). Hirst, J. Chem. Soc. 1942, p. 77 reported on the structure of fenugreek mucilage. He tried to separate it into a galacton and a mannqn but was not successful.

Recent work in the literature on this group of mucilages has indicated that they are true manno-galactons. The reports by earlier

workers that d-glucose and fructose were present is probably due to the presence of impurities and to faulty methods of testing for fructose.

Difficulty in Testing Qualitatively and Determining  
Quantitatively the Various Sugars in the Presence of Each Other.

It has long been known that the methods used in testing qualitatively and determining quantitatively the various sugars in a mixture such as that from the hydrolysis of an endosperm mucilage are very inexact. This is especially true in the qualitative tests for levulose and to quantitative determination of d-galactose. Kruisber, (Browne and Zerban pp. 712-713) has investigated the qualitative tests for levulose and for pentoses.

Experimental Work on the Composition of the Endosperm  
Mucilages.

Our first experimental work on the qualitative composition led us to report the absence of fructose. Later, however, one of our graduate students repeatedly tested the sugars obtained by hydrolysis of various endosperm mucilages for fructose by the Seliwanoff reagent and reported that he always obtained a positive reaction. Dr. Wise of the Institute Staff stated that he obtained this test on pure sugars which had been heated for some hours with dilute sulfuric acid. Dr. Wise suggested that this point be carefully re-investigated. It seems well to describe the methods used in determining the composition of the endosperm mucilages.

### The Preparation of the Endosperm Mucilages.

The endosperms are first isolated and the fairly pure mucilages are prepared from these, as described in previous reports.

### The Hydrolysis of the Mucilages and the Isolation of Mannose and Galactose.

In most of the previous work we have mixed the mucilage with a 4% solution of sulfuric acid and heated the mixture in a bath of boiling water for approximately 15 hours. The sulfuric acid was removed by treating the solution with excess barium carbonate and filtering off the barium sulfate. The filtrate was concentrated down and the gum sugar dissolved in ethanol and filtered from any insoluble material. The ethanol was concentrated and crystalline d-mannose and d-galactose were isolated by use of glacial acetic acid. These sugars were identified by their specific rotations and by conversion to mannose phenyl hydrazone and to galactose osazone. These two sugars always found present in the endosperm mucilages from seeds of legumes.

### Tests for Fructose

Qualitative tests for a ketohexose on the sugars obtained by hydrolysis of the mucilages with a 2% solution of sulfuric acid were always positive, indicating the presence of such a sugar. However, I was never able to isolate any of the sugar. Specific rotations of the gum sugar after removal of much of the galactose

and mannose were always positive. If any appreciable amount of fructose had been present the rotation should have been strongly levo. I was in doubt as to the presence or absence of fructose. This point was cleared up as described later.

#### The Absence of Uronic Acids

The naphthoresorcinol test of Tollens was repeatedly made on the mucilages and on the products of hydrolysis of these substances. The test was always negative. No barium salt of a uronic acid was ever obtained after hydrolysis of the mucilages. When the mucilages were heated with a 12% solution of hydrochloric acid only traces of carbon dioxide were obtained. These tests prove the absence of uronic acids.

#### The Absence of Pentosan and Methyl pentosan Materials

The sugars obtained by hydrolysis of the mucilages were repeatedly tested for pentoses by the orcinol reagent and by the aniline acetate test. The tests were always negative but this might have been due to the presence of such large amounts of hexoses. This point was cleared up by running regular pentosan determinations on the pure mucilages. Weighed amounts of the pure mucilages were placed in distilling flasks together with 12% hydrochloric acid and distilled as described in the pentosan determination, A.O.A.C. The distillates were treated with phloroglucinol reagent. In no case did the distillate from a pure mucilage give any furfural phloroglucide. This proved the absence of pentosan, methyl pentosan and uronic acids from the mucilages.

On June 5th, 1943, when I reached the Institute, my work on the qualitative composition of the endosperm mucilages had reached this point. I was sure of the presence of mannose and galactose and the absence of pentosan, methyl pentosan, but was in doubt as to the presence of fructose and glucose.

On conferring with Dr. Wise of the Institute Staff, he questioned the reliability of the Seliwanoff test. He also suggested that a 2% solution of sulfuric acid be used in place of the 4% acid which we had been using and that the period of heating be shortened to 6 hours. As a result of this conference certain experiments were planned which would settle the points in question. These experiments are given in my Research Notebook No. 503 under Project 849 and are numbered experiments 1 to 5.

Experiment No. 1, on p. 7 of book 503 was designed to test the reliability of the Seliwanoff test for ketohexoses and to determine the conditions under which it can be regarded as positive. Into each of four large test tubes were placed 50 cc. of a 2% sulfuric acid solution. To tube A was added 1 g. of pure d-galactose; to tube B was added 1 g. of pure d-mannose; to tube C was added 1 g. pure d-galactose + 1 g. pure d-mannose; to tube D was added 1 g. each of pure d-galactose, d-mannose, and d-fructose. The four tubes were then heated for 6 hours in a bath of boiling water. The contents of tubes A, B, C, remained clear while those in tube D turned a brownish yellow and a precipitate settled out. The four solutions were filtered and each subject to the Seliwanoff test. Solutions A, B, and C, gave a red

coloration with the Seliwanoff reagent while solution D gave an intensely red colored solution with the reagent. On the other hand solutions of pure d-mannose and d-galactose which had not been heated with an acid gave just a trace of red coloration.

This experiment proves that in order for the Seliwanoff test to be regarded as positive for a ketohexose, where the solution has been heated with an acid, the test must be intensely colored, not just an ordinary pale red color.

I had brought along with me the gum sugars obtained by the hydrolysis of about a dozen endosperm mucilages at Tucson. These were now tested with the Seliwanoff reagent and carefully compared with the tests made on tubes A, B, C, and D. above. All of the gum sugars gave red colorations that appeared like that from tube C. None were colored nearly so deeply as the control solution from tube D. This at once indicates the absence of a ketohexose in the mucilages.

Experiments No. 2 and No. 3 on pages 15 and 20 of research notebook No. 503 were designed to test directly for fructose in some twenty endosperm mucilages prepared from various seeds. For details of the experiments reference should be made to the notebook. In brief the experiments consisted in preparing known mixtures of (A) d-mannose and d-galactose; (B) d-mannose and d-galactose and a trace of d-fructose, and heating them in a bath of boiling water with a 2% solution of sulfuric acid for 6 hours. At the same time 0.1 g. of

the various mucilages were placed separately in test tubes and heated for the same length of time with 2% sulfuric acid.

After removal of the sulfuric acid the various solutions were carefully compared. Solution B was colored a brownish yellow. All of the solutions from pure endosperm mucilages which had originally been prepared from the free endosperms and not from the whole seed, were water clear. This was also true of solution A. The Seliwanoff test was now applied to all of these solutions. The solutions from all of the mucilages gave colors that corresponded exactly with solution A. From the amounts of fructose present in solution B it was evident that the mucilages must have contained less than 2% fructose. Since they gave colors that corresponded exactly with a known solution of mannose and galactose containing no fructose, I must conclude that none of the mucilages examined contained any fructose. See Research Notebook No. 503 pp. 24-25.

From the above experiments we may reasonably conclude that the endosperm mucilages obtained from seeds of legumes do not contain any fructose. There is still the possibility that these mucilages may contain d-glucose. In fact some of the early investigators reported the presence of d-glucose.

Experiments 4 and 5, pages 28 and 36 of research Notebook No. 503 were derived to test for the presence of d-glucose in the pure endosperm mucilages. These experiments use the method devised by

This experiment bears out the statement of Daoud that after removal of the mannose as the hydrazone, the osazone of pure d-galactose is obtained. It proves that in the endosperm mucilages there can be no more than traces of d-glucose present.

It thus appears that the pure endosperm mucilages obtained from seeds of various legumes are really manno-galactons and do not contain other sugars or uronic acids.

ea/jvl

Daoud, [Biochem. J. 26, 255, (1932)] in proving the absence of d-glucose from the mucilage obtained from fenugreek. Reference should be made to the notebook for details of the experiment.

In each of some 25 test tubes were placed known volumes of a 2% sulfuric acid solution. Tube No. 1 contained 1 g. d-galactose.

Tube No. 2 contained 1 g. d-mannose.

Tube No. 3 contained 0.7 g. mannose  
+ 0.3 g. galactose.

Tube No. 4 contained 0.17 g. mannose  
+ 0.2 g. galactose + 0.1 g. fructose.

The other tubes contained known weights of specific pure endosperm mucilages. The tubes were heated for 6 hours in a bath of boiling water. The sulfuric acid was removed with barium carbonate and the mannose in each tube was removed in the regular way as mannose phenyl hydrozone.

The sugars in the filtrates from the mannose phenylhydrozones were converted to the osazones. These were filtered off and dried and melting points were determined on each. A trace of osazon was obtained from tube No. 2. Some osazone was obtained from tube No. 4. These two osazones melted between 203 and 204° C. This is the melting point of the osazone of d-glucose. The osazones from all of the other solutions melted at 186° to 188° C. which is the melting point of the osazone of d-galactose. I had expected the osazone from No. 4 to show an intermediate melting point.

PROJECT REPORT FORM

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PAGE 66720 TO 66721  
SIGNED N. A. Kjelson

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Dr. Rowland  
Mr. Kjelson

N. A. Kjelson

Subject:

Comparison of the cold water soluble fraction (CWS) of honey locust gum G11 with the cold water insoluble (CWI) fraction as paper pulp deflocculating agents and paper strengthening materials.

Discussion:

The CWS fraction (GMI code number 109940) and the CWI fraction (GMI code number 109941) were received as white powders, the former being of a "fibrous" type. To put them on a comparable basis, both were cooked to 80° C. at 0.50% solids and cooled before making viscosity measurements and applying for paper tests.

The Ostwald pipette viscosities of the cooked gums at 0.50% solids at 30.0° C. were as follows:

	Seconds	Relative Viscosity
CWS fraction 109940	360.4	68.648
CWI fraction 109941	191.4	36.457

The sheets prepared with these gums in varying amounts were prepared according to a standard procedure described in previous reports. The sheets tested for wet tensile strength were first dipped into 1.0% borax solution to set-up the gum to a water-resistant adhesive and then redried.

The test results are given in the accompanying table.

It can be seen that both samples show excellent properties in paper and both in about the same magnitude for each of the tests made here. The real differences between the samples cannot be read from this table of data.

since an insufficient number of samples were run to obtain a small enough probable error for two materials which are so similar in their characteristics. However, the differences in effectiveness between the two samples is of no practical significance since an economical separation of this type could not, in all probabilities, be made on a commercial scale.

nak/esb

TABLE I

## SHEET PROPERTIES OF THE CWS AND CWI GUMS

	Thwing Number	Formation % Inc. over Blank	Bursting Points	Strength % Increase over blank	Wet Tensile Strength after Borax Dipping Units	% Increase over Blank
1% CWS fraction of G11	37.1	41.6	29	71.6	10.1	431.6
2% CWS fraction of G11	38.8	48.1	34.1	101.8	13.1	589.5
4% CWS fraction of G11	42.8	63.4	38.0	134.9	17.4	815.8
1% CWI fraction of G11	36.2	38.2	29.1	72.2	10.5	452.6
2% CWI fraction of G11	40.0	52.7	31.6	87.0	12.7	568.4
4% CWI fraction of G11	44.0	67.9	41.4	145.0	16.9	789.5
Blank	26.2		16.9		1.9	

# PROJECT REPORT FORM

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PAGE 66724 TO 66728  
SIGNED N. A. Kjelson

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N. A. Kjelson

Subject:

Paper making properties of pentosan pulps prepared by Mathieson Chemical Company.

## Procedures and results:

Two samples of pentosan pulps "said to increase the rate of hydration of paper pulp in the beater" were received. The pulps were numbered #1363-6 and #1368-1, the former having the appearance of unbleached spruce sulfite pulp. Samples were dried to constant weight at 105° C. for moisture determinations. #1363-6 showed 35.54% oven dry solids and #1368-1 showed 16.62% oven dry solids. These pentosan pulp samples were compared with locust bean gum in both a free and a slow stock for their comparative properties in paper as adhesive, deflocculation and wet strength agents.

Weyerhaeuser bleached sulfite was again used as the base pulp but two different beating periods were used: a ten minute interval and a 30 minute interval. Sample #1363-6 was full of dark shives which, if added undispersed, might spot the sheet and interfere with the formation readings. The pentosan pulps were, therefore disintegrated in the Waring Blender for 5 minutes at 1% solids before addition to the pulps. The locust bean gum was prepared for addition by cooking at 80° C. in a water bath and cooling to room temperature.

Freeness curves (Schopper-Riegler) were run on the pulps containing the various gums in various amounts. This was done to note the depression in the freeness caused by the addition of the gum. The test results are shown in the accompanying tables I to IV inclusive.

The conclusions are self evident, being that the pentosan pulps described have no desirable properties as papermaking materials.

TABLE I

SCHOPPER-RIEGLER FREENESS OF PULPS CONTAINING  
THE HEMICELLULOSE MATERIALS, IN CUBIC CENTIMETERS

	8% Addition	4% Addition	2% Addition
Pulp beaten 10 minutes			
Pentosan Pulp #1363-6	835	840	850
Pentosan Pulp #1368-1	820	845	850
Locust Bean Gum	840	835	840
Pulp only	850		
Pulp beaten 30 minutes			
Pentosan Pulp #1363-6	645	655	660
Pentosan Pulp #1368-1	600	630	650
Locust Bean Gum	530	530	540
Pulp only	675		

TABLE II

BURSTING STRENGTHS OF SHEETS CONTAINING

THE HEMICELLULOSE MATERIALS, IN POUNDS PER IN.<sup>2</sup>

	8% Addition		4% Addition		2% Addition	
	Pounds	% Increase over untreated sheet	Pounds	% Increase over untreated sheet	Pounds	% Increase over untreated sheet
Pulp Beaten 10 Minutes						
Pentosan Pulp #1363-6	16.9	--	19.8	--	17.3	--
Pentosan Pulp #1368-1	23.5	8.80	19.6	--	19.3	--
Locust Bean Gum	33.6	55.6	34.2	58.3	33.8	56.5
Pulp only	21.6					
Pulp Beaten 30 Minutes						
Pentosan Pulp #1363-6	40.0	1.52	40.7	3.30	40.4	2.54
Pentosan Pulp #1368-1	40.7	3.30	39.9	1.27	38.9	--
Locust Bean Gum	55.7	41.4	53.6	36.0	52.2	32.5
Pulp only	39.4					

TABLE III

THWING FORMATION NUMBERS OF SHEETS  
CONTAINING THE HEMICELLULOSE MATERIALS

	8% Addition	4% Addition	2% Addition
Pulp Beaten 10 Minutes			
Pentosan Pulp #1363-6	25.2	25.5	25.6
Pentosan Pulp #1368-1	26.8	26.4	26.0
Locust Bean Gum	43.5	44.5	44.3
Pulp only	22.1		
Pulp Beaten 30 Minutes			
Pentosan Pulp #1363-6	28.2	28.6	28.2
Pentosan Pulp #1368-1	27.6	31.1	27.3
Locust Bean Gum	47.9	45.1	47.0
Pulp only	27.5		

TABLE IV

WET TENSILE STRENGTHS OF SHEETS CONTAINING  
THE HEMICELLULOSE MATERIALS AND BORAX, IN UNITS

	3% Addition % Increase over untreated sheet	Units	4% Addition % Increase over untreated sheet	Units	2% Addition % Increase over untreated sheet	Units
Pulps Beaten 10 Minutes						
Pentosan Pulp #1363-6	--	1.3	7.15	1.5	1.7	21.4
Pentosan Pulp #1368-1	35.7	1.9	14.3	1.6	1.4	--
Locust Bean Gum Pulp only	850.0	13.3	757.1	12.0	10.8	671.4
Pulps Beaten 30 Minutes						
Pentosan Pulp #1363-6	--	2.2	--	2.0	2.5	8.70
Pentosan Pulp #1368-1	43.5	3.3	4.35	2.4	2.4	4.35
Locust Bean Gum Pulp only	808.7	20.9	726.1	19.0	17.9	678.3