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PREPARATION OF ENZYMES CAPABLE OF CONVERTING MANNOGALACTAN MUCILAGES FOR USE AS TUBSIZING AND COATING ADHESIVES

## INTRODUCTION

It has been shown that converted mannogalactan mucilages can be made which possess properties desirable for tubsizing and coating adhesives (see Reports 1, 5, 6, 9, 11, and 13). The methods used to convert or reduce the potential viscosity of these products—namely, chlorination, acid hydrolysis, and dextrinization may be somewhat uneconomical at the present price of the mucilage and therefore, a cheaper method was sought, which might find immediate use. This led to an investigation of the possibilities of using enzymes in a manner similar to that used by the industry for conversion of starch for tubsizing, calender sizing, and coating adhesives. The feasibility of using enzymes for this purpose was shown in a preliminary way by the work of Report Six in which the enzyme of sprouted guar seed was used.

A number of commercial enzyme products were tried as converting agents for the mucilage. Most of these were found to be inactive in the presence of mannogalactan and the few which promoted an active hydrolysis did so only when relatively large amounts of enzyme were used. The high prices of the active enzymes precluded their commercial use. Among those tried may be mentioned Katozyme, Clarase, Sizyme, Takadiastase, Amyliq, Vanzyme, Liquidase PL-100, Enzyme 1275 (Takamine), Pancreatin, Diastases A and C, and pectinols A, W, and M.

To the best of our knowledge, no commercial enzyme is produced in the United States for the express purpose of converting mannogalactan mucilages. There are indications in the literature that at least one is or has been on the European market.* This product is made in Switzerland and is called "Helisol." It is said to be made from germinated mannogalactan containing seeds and is very active. Also some mention is made of another source from snails but this is probably not a commercial product. The available literature has been reviewed in Report Six.

The above information led to attempts to produce the necessary enzymes from organisms in a manner similar to that used for manufacturing starch modifying enzymes.

Commercial starch hydrolyzing enzymes are obtained from three principal sources--namely, higher plants (mostly seeds), microbes, and animal glands. A wide variety of amylases may be obtained from these sources and they differ considerably in properties such as thermostability, type of hydrolysis, and optimum conditions of action.

The use of guar seed as a source of enzyme for converting mannogalactan mucilage would necessitate the germination of considerable

* Tagliani, Melliand Textilberichte, 11, 458(1930).

quantities of seed and may be uneconomical at the present time unless quantities of cull seed were available. Animal gland enzymes did not appear promising as revealed by preliminary testing of the commercial products. The remaining source--namely, microbes appeared to invite investigation inasmuch as a wide variety of enzymes may be obtained from various organisms. Furthermore, the starch converting enzymes obtained from certain bacteria appear to be of a particularly desirable type. The experiments of this report have therefore, centered around this source for producing mannogalactan converting enzymes.

## EXPERIMENTAL

### Preliminary Experiments with Mold Enzymes

It seemed reasonable to assume that organisms capable of existing on mannogalactan mucilage as a substrate might be found clinging to various seeds of this type. On this basis several varieties of seed were placed in crystallizing dishes, dampened with water, covered with watch glasses and placed in an oven at 37° C. After six days most of the germinating seeds showed evidence of mold growth but one in particular, flame tree seed, appeared to possess the best growths. There were predominantly two colored types of growth present, a greenish blue mold and a dark brown mold. A consultation with Dr. Appling was held which resulted in his removing some of the spores of each type and transferring to malt-agar slants. After repeated subculturing of the spores, the types appeared to be relatively pure and were then transferred to 5% mannogalactan slants made from guar G4-2. Two days later the tubes were observed and the organisms were growing and appeared to have liquified a shallow layer of the mannogalactan. One tube of each organism was mixed with a sterile loop in order to determine whether or not sufficient enzyme was present to liquify the entire slant. After two hours, the entire slant in both cases had been liquified. The contents of these tubes were centrifuged and one-ml. aliquots of the supernatant liquid were mixed with fresh 5% mannogalactan slants. Within two hours, the extract from the brown mold had liquified the substrate. The green mold extract did not appear to be as potent but a considerable part of the mucilage had liquified. Further work with the green mold was discontinued in favor of the brown species.

Larger enzyme cultures were next made by adding 120 grams of 5% cooked G4-2 mucilage to one-liter Erlenmeyer flasks, sterilizing and inoculating with a sterile water suspension of the brown mold spores. These cultures were incubated at 30° C. After four days, the entire substrate had been liquified and the liquid was poured off and centrifuged. The enzyme activity was found to be easily as potent as that present in the test tube slants. Attempts to concentrate the extract by acetone precipitation and vacuum distillation were not successful. Serious inactivation occurred in the former case and persistent foaming prevented the latter procedure although this was overcome in later experiments. The next problem was to devise a more accurate method for measuring the relative enzyme potency.

DEvelopment of a Method for Evaluation of the Enzyme Potency

Since viscosity is a prime consideration in the conversion of mannogalactan mucilages for tubsizing and coating purposes, it seemed expedient to use a viscosity method for measuring enzyme activity. During the early stages of the problem, it was believed that boric acid would probably be detrimental to enzyme action and the borax method of cooking seemed undesirable. Considerable difficulty has been experienced in cooking the guar mucilages without borax because of doughball formation. Doughballs would be expected to influence the viscosity determinations so it was decided that locust bean gum would be used for the substrate since no such difficulty occurs with this mucilage. Numerous experiments resulted in the following procedure for locust bean gum.

Method of Cooking

Ten grams of locust bean gum were added with stirring to 1500 ml. of water in a tared 2-liter beaker. The temperature was raised rapidly by direct steam injection to 90-93° C. during seven minutes time and then held there for 5 minutes. The mixture was diluted to 0.5% mucilage, placed in two, two-liter flasks, weighed, and autoclaved at 120° C. for 30 minutes with additional water and four 500-ml. Erlenmeyer flasks equipped with rubber stoppers. After sterilization the mucilage was brought to 0.5% concentration again with sterile water, poured into sterile flasks, stoppered and allowed to stand at room temperature until used. The viscosity of mucilage prepared in this manner remained constant for several months and the same viscosity within 2 or 3 seconds could be obtained from batch to batch when the time intervals of heating, stirring and autoclaving were held constant. Particularly important is the time of stirring from the time the mucilage is added until the temperature reaches 90° C. Apparently there is sufficient native enzyme in locust bean gum to change the viscosity. The major part of this enzyme is inactivated at 90° C.

Procedure for Measuring Enzyme Activity

One hundred grams of 0.5% locust bean gum were weighed into a 250-ml. Erlenmeyer flask. Then 56.6 g. of water and 0.15 ml. of 1% acetic acid were added and the flask was placed in a water bath at 30° C. After 15 minutes, 10 ml. of 0.5% enzyme solution (0.5 ml. in 100 ml. of solution) were added and a clock started. Then 10 ml. of the mixture were pipetted rapidly to an Ostwald viscometer at 30° C. and viscosity measurements were made at 10, 20, and 30-minute intervals.

A blank without enzyme was run in each of the viscometers in order to obtain the viscosity at zero time. The difference between the viscosity of the enzyme solution and water was insignificant in most cases. The viscometers were chosen so that the constantswere as nearly the same as possible. In the later work, the viscometer times of outflow with 10 ml. of water varied from 4.28 to 4.36 seconds. Since no mathematical relationship between enzyme activity, concentration and time has been worked out, thus far, the activity has been tentatively measured as the per cent of the original viscosity attained in 20 minutes time at 30° C.

A considerable amount of difficulty was experienced when guar mucilages such as G4-5 were sterilized by the above technique. Guar appears to be more heat labile than locust bean gum and therefore, the viscosity of the autoclaved mucilage was much too low for measurement purposes--being about 58 seconds at 0.75%. Adjustment of the pH to the neutral point did not seem to prevent this breakdown. The viscosity from batch to batch did not check very well and even after sterilization the viscosity continued to change from day to day. As the work progressed and it became necessary to use a guar mucilage for determining the activity, it was found best to cook fresh batches of G4-5 each day and check the viscosity in both morning and afternoon.

G4-H mucilage was also found to be quite heat labile and attempts to sterilize this mucilage were unsuccessful. However, the purity of this product enabled the use of a simpler procedure which was found to be quite suitable.

Ten grams of G4-H were sifted into 1500 ml. of distilled water in three minutes. Steam was injected and the temperature raised to 93° C. in 8 minutes where it was held for 10 minutes. The mixture was diluted to 0.5% concentration and poured into clean, dry 500-ml. Erlenmeyer flasks and stored at room temperature. The viscosity of this material remained constant for 2 to 3 days.

Preliminary Experiments with Pectinol W. and
Several Acetone Precipitated Enzymes

Qualitative experiments had indicated that Pectinol W enzyme (Röhm and Haas) acts fairly rapidly on locust bean gum when used in rather large amounts. The experiments of Table I were an attempt to determine whether or not this enzyme could be used in commercially feasible quantities for converting purposes. The work showed that Pectinol W probably is not sufficiently active to be used commercially.

More precise determinations of the activity of the several mold enzymes which had been tested earlier were not at first very encouraging as shown in Table II. Concentration of the enzymes by acetone precipitation or evaporation with starch seemed to seriously inactivate the products. It was decided that further work would be done with the

TABLE I

EXPERIMENTS WITH THE ACTION OF PECTINOL W ON LOCUST BEAN GUM

| Ex. No. | Amount Enzyme Added % on Mucilage | Buffer Kind | Buffer Ml. | Temp. °C. | pH of Mixture | Activity* on LBG | Remarks and Notes |
|---|---|---|---|---|---|---|---|
| 1 | 1 | HAc | 9.0 | 30 | 3.51 | 81.7 | |
| 2 | 1 | HAc | 0.15 | 30 | 5.08 | 83.3 | Notes on Buffer Solutions |
| 3 | 1 | $NaHCO_3$ | 0.65 | 30 | 7.1 | 88.2 | |
| 4 | 1 | Oxalic | 0.9 | 30 | 3.63 | 80.3 | Acetic acid, 1% by volume |
| 5 | 2 | HAc | 9.0 | 30 | 3.57 | 69.1 | $NaHCO_3$, 1% by volume |
| 6 | 5 | HAc | 9.0 | 30 | 3.57 | 51.8 | Oxalic acid, 1% by volume |
| 8 | 1 | Oxalic | 6.5 | 30 | 2.46 | 79.7 | |
| 9 | 1 | HAc | 0.15 | 40 | 5.05 | 83.1 | Pectinol W, 0.05% solution by volume |
| 10 | 1 | HAc | 9.0 | 40 | 3.42 | 80.6 | |
| 11 | 1 | Oxalic | 6.5 | 40 | 2.45 | 87.8 | |
| 12 | 1 | HAc | 0.15 | 50 | 5.0 | 73.5 | |
| 13 | 1 | HAc | 9.0 | 50 | 3.55 | 74.4 | |
| 14 | 1 | Oxalic | 6.5 | 50 | 2.63 | 83.9 | |
| 15 | 1 | HAc | 0.15 | 60 | 5.1 | 75.3 | |
| 16 | 1 | HAc | 9.0 | 60 | 3.63 | 72.2 | |
| 17 | 1 | Oxalic | 6.5 | 60 | 2.60 | 81.4 | |
| 18 | Blank | Oxalic | 6.5 | 60 | 2.60 | 92.0 | |
| -- | 1 | HAc | 0.15 | 30 | 5.07 | 82.8 | Guar G4-H as substrate |

* Activity is defined as the per cent of the original viscosity present after 20 minutes action of the added enzyme. Therefore, by definition, the lower the activity number, the more potent is the enzyme.

TABLE II

EXPERIMENTS WITH SEVERAL MOLD ENZYMES

| | | | Incubation Conditions | | | Determination of Enzyme Activity | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Enzyme | Substrate Comp. or No. | Inoculant | Temp. °C. | Time in hr. | pH of Enzyme | Ex. No. | Amount Enzyme Added % on Mucilage | Buffer Kind | Buffer Ml. | pH | Activity at 30° C. on LBG | Remarks |
| E54-603 | 5% G4-2 | Brown mold | 25 | 38 days | 2.7 | 22 | 1.0 | $NaHCO_3$ | 0.65 | 6.75 | 74.7 | Enzyme liquid evaporated down |
| | | | | | | 23 | 1.0 | HAc | 0.15 | 4.69 | 79.5 | To dryness with starch |
| | | | | | | 24 | 1.0 | HAc | 9.0 | 3.51 | 80.1 | |
| E99-3-631 | 5% locust bean gum | Brown mold | 37 | 24 | 3.72 | 28 | 1.0 | $NaHCO_3$ | 0.65 | 7.08 | 87.1 | Acetone precipitated enzyme |
| | | | | | | 29 | 1.0 | HAc | 0.15 | 5.14 | 87.3 | |
| | | | | | | 30 | 1.0 | HAc | 9.0 | 3.54 | 89.3 | |
| E100-1-631 | 5% LBG | Brown mold | 37 | 120 | 3.02 | 37 | 20.0 | HAc | 0.15 | 5.06 | 66.4 | Liquid enzyme used |
| | | | | | | 39 | 200.0 | HAc | 0.15 | 4.8 | 52.9 | |
| | | | | | | 49 | 200.0 | HAc | 0.15 | 4.72 | 69.3 | |
| E66-1-603 | 6% G4-2 | 2 ml. of Brown mold | 30 | 120 | 4.85 | 41 | 1.0 | HAc | 0.15 | 5.03 | 81.2 | Evaporated to dryness with starch |
| E66-2-603 | 6% G4-2 | 5ml. of Brown mold | 30 | 120 | 4.19 | 44 | 1.0 | HAc | 0.15 | 5.03 | 69.8 | Evaporated to dryness with starch |
| E99-4-631 | 5% LBG | Aspergillus niger | 37 | 48 | -- | 42 | 1.0 | HAc | 0.15 | 5.05 | 91.5 | Alcohol precipitated enzyme |
| Liquidase PL-100 | -- | -- | -- | -- | -- | 49 | 1.0 | HAc | 0.15 | 5.20 | 88.3 | Commercial enzyme |
| E99-1-631 | 5% LBG | Brown mold | 37 | 48 | -- | 38 | 1.0 | HAc | 0.15 | 5.02 | 65.4 | Mycelialenzyme |

TABLE III

SUMMARY OF SUBSTRATE COMPOSITIONS USED IN FOLLOWING EXPERIMENTS

| Substrate No. | Mucilage Kind | Mucilage Amt. g. | Volume 2% Peptone ml. | Nutrient Salt Solution No.** | Nutrient Salt Solution Amt. ml. | $CaCO_3$ Added, g. | Water Added, ml. | Other Additions |
|---|---|---|---|---|---|---|---|---|
| 1 | LBG* | 2.5 | — | - | — | - | 50 | — |
| 2 | LBG | 2.5 | 25.0 | - | — | - | 25 | — |
| 3 | LBG | 2.5 | 25.0 | 1 | 25 | - | — | — |
| 4 | LBG | 2.5 | 25.0 | 1 | 17 | - | — | 6 ml. 4% $CaCl_2$ |
| 5 | LBG | 2.5 | 25.0 | - | — | 1.0 | 25 | — |
| 6 | G4-2 | 2.5 | 25.0 | 1 | 25 | 1.0 | — | — |
| 7 | G4-2 | 2.5 | — | - | — | 1.0 | 50 | — |
| 8 | G4-5 | 2.5 | 25.0 | 1 | 25 | - | — | — |
| 9 | G4-5 | 2.5 | 2.0 | 1 | 25 | - | 23 | — |
| 10 | G4-5 | 2.5 | 25.0 | 2 | 25 | - | — | — |
| 11 | G4-5 | 2.5 | 25.0 | 2 | 25 | - | — | 0.5 gilactose |
| 12 | G4-5 | 1.25 | | | | | | |
| | LBG | 1.25 | 25.0 | 2 | 25 | - | — | — |
| 13 | G4-2 | 2.5 | 25.0 | 2 | 25 | - | — | — |
| 14 | G4-5 | 1.5 | 15.0 | 2 | 10 | - | 295 | — |
| 15 | G4-5 | 2.5 | 25.0 | 2 | 25 | 1.0 | — | |
| 16 | G4-5 | 7.5 | 75.0 | 2 | 75 | 1.0 | — | — |
| 17 | G4-5 | 0.8 | 8.0 | 3 | 8 | - | — | — |
| 18 | G4-5 | 7.5 | 75.0 | 3 | 75 | 1.5 | — | |
| 19 | G4-5 | 7.5 | 75.0 | 3 | 75 | - | — | — |
| 20 | G4-5 | 7.5 | 75.0 | 2 | 75 | - | — | — |

* LBG - locust bean gum
** For composition of nutrient salt solution, see next page.

COMPOSITION OF NUTRIENT SALT SOLUTIONS
REFERRED TO IN TABLE III

No. 1: 2.5 g. $Na_2HPO_4 \cdot 12H_2O$
1.0 g. $MgSO_4 \cdot 7H_2O$
0.2 g. KCl
0.8 g. $FeSO_4$
Water to make 1000 cc.
pH = 6.75

No. 2: 2.5 g. $Na_2HPO_4 \cdot 12\ H_2O$
1.0 g. $MgSO_4 \cdot 7H_2O$
1.0 g. KCl
Water to make 1000 cc.

No. 3: 2.5 g. $Na_2HPO_4 \cdot 12\ H_2O$
0.4 g. $(NH_4)_2SO_4$
0.8 g. $MgSO_4 \cdot 7H_2O$
1.0 g. KCl
0.2 g. $CaCl_2$ (anhy.)
0.05 g. $FeSO_4 \cdot 7H_2O$
0.05 g. $MnCl_2 \cdot 4H_2O$
Water to make 1000 cc.

liquid enzyme as isolated from the culture by centrifugation. It was soon found that the activity of these preparations could be maintained for periods of several days if they were kept saturated with toluene and placed in a refrigerator at 10° C.

Experiments with Bacterial Enzymes

During a series of experiments with an old culture of the Brown mold, a new organism began to grow at the expense of the mold. It seemed to possess a rather potent enzyme and further experiments were made to determine its source. It now appears that the mold culture was contaminated with an unknown organism. Upon adding peptone and certain nutrient salts to the mucilage substrate, the new organism growth was exceedingly prolific and the enzyme potency of the centrifuged liquor was particularly outstanding. Addition of peptone alone to the locust bean gum gave an increase in enzyme potency but the nutrient salt mixture increased the potency many times, see Table IV.

Further experiments with the original culture of pure Brown mold gave evidence that it, too was contaminated with the new organism and therefore, a pure mold had never actually been used. The lack of proper nutrient materials had probably heretofor prevented rapid growth of the contaminating organisms.

Microscopic examination of the enzyme cultures showed what appeared to be a mixture of bacillus type organisms. Mr. McCoy of the

TABLE IV

EXPERIMENTS ON GROWTH CONDITIONS OF THE BROWN MOLD

| | | | Incubation Conditions | | | Determination of Enzyme Activity | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Enzyme No. | Substrate Comp. No. | Inoculant | Temp. °C. | Time in hr. | pH of Enzyme | Ex. No. | Amount Enzyme Added, %* | pH | Activity at 30° C. | Remarks |
| 1 | 1 | 5 ml. | | | | | | | | no mold growth |
| | | brown mold | 37 | 40 | 5.35 | 52 | 200 | 5.00 | 75.3 | Spores from E52-603 |
| | | | 37 | 113 | 4.8 | 63 | 200 | 5.13 | 72.2 | No mold growth |
| 2 | 2 | 5 ml. brown mold | 37 | 40 | 5.65 | 53 | 200 | 5.10 | 41.3 | No mold growth |
| 3 | 3 | 5 ml. brown mold | 37 | 42 | 7.35 | 56 | 200 | 5.50 | 10.9 | No mold growth |
| | | | 37 | 65 | 8.2 | 60 | 200 | 6.02 | 10.6 | |
| | | | 37 | 113 | 8.45 | 65 | 200 | 5.62 | 9.3 | |
| | | | | | | 93 | 200 | 5.61 | 18.0 | Enzyme and toluene preserved 5 days |
| 4 | 4 | 5 ml. brown mold | 37 | 42 | 4.45 | 55 | 200 | 4.8 | 39.8 | Much mold growth |
| | | | 37 | 116 | 5.1 | 70 | 200 | 4.97 | 14.8 | |
| 5 | 5 | 5 ml. brown mold | 37 | 44 | 6.8 | 59 | 200 | 5.4 | 52.2 | No mold growth |
| | | | 37 | 96 | 6.6 | 73 | 200 | 5.41 | 56.3 | No mold growth |
| 6 | 1 | 5 ml. brown mold | 37 | 44 | 5.65 | 61 | 200 | 5.02 | 74.6 | No mold growth |
| | | | 37 | 96 | 4.65 | -- | - | -- | — | No mold growth |
| 7 | 6 | Pure culture brown mold | 37 | 70 | 7.35 | 74 | 200 | 5.25 | 14.1 | Much mold growth |
| | | | 37 | | | 82 | 10 | 5.11 | 30.4 | |
| 8 | 7 | Pure brown mold | 37 | 70 | 7.0 | | 200 | 5.25 | 23.0 | Much mold growth |
| 9 | 3 | Old brown mold | 37 | 48 | 5.9 | | 200 | 5.37 | 57.2 | Much gas |

* This is per cent of liquid enzyme as centrifuged from the culture. Thus one ml. of the liquid was added to the solution containing 0.5 g. of mucilage.

microbiology department made streak cultures of enzyme culture No. 3 on nutrient agar plates attempting thereby to isolate colonies of the various organisms present. Following this, a determination of the organism responsible for the best enzyme production could be made. As the isolation proceeded, it became evident that the original contaminant did not consist of a large number of organisms but rather very few. Inoculation of a locust bean, peptone, salt substrate was made from a plate culture of the original enzyme culture No. 3 and called No. 18. This culture (No. 18) showed very potent enzyme activity, see Table VI. As further purification of the unknown organism took place, it appeared that about five types of colonies were growing. These were transferred to nutrient agar slants and their relative enzyme potencies were determined by growing upon locust bean gum, peptone, and nutrient salt mixtures. These experiments are given in Table VII, where it is evident that the organisms developed at about the same rate, produced the same enzyme potency and destroyed the enzyme at approximately the same rate. There may be some question about the No. 4 organism but this has not as yet been checked. These facts indicated that the 5 types of colonies may be variants of the same organism. Mr. McCoy could not distinguish between them by staining techniques or nutritional behavior and subsequent testing of the enzymes produced have not indicated significant differences. Until such time as the organism can be identified, it will be called Bacillus X. The steps taken in the isolation are given in Flow Chart I and the description of colonies are in Table VIII.

TABLE V

EXPERIMENTS WITH THE UNKNOWN BACILLUS X

| Enzyme No. | Substrate Comp. No. | Inoculant | 37° C. Incubation Time in hr. | pH of Enzyme | Determination of Enzyme Activity: Ex. No. | Amount Enzyme Added, ml. | pH | Activity | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 3 | 5 ml. culture from No. 3 | 46 | 8.45 | 68 | 200 | 6.5 | 9.6 | Heavy pellicle |
| | | | 93 | 8.22 | 89 | 10 | 5.22 | 49.1 | Aged at room temp. |
| 11 | 3 | Pure brown mold | 48 | 8.15 | 72 | 200 | 6.22 | 13.3 | Pellicle formed |
| | | | | 8.12 | 86 | 10 | 5.22 | 35.4 | |
| 12 | 3 | Plate culture of No. 3 | 18 | 5.9 | 77 | 200 | 5.22 | 8.6 | Heavy pellicle |
| | | | 20 | 5.9 | 79 | 20 | 5.20 | 12.8 | |
| | | | 20 | 8.35 | 83 | 10 | | 21.4 | |
| | | | 41 | 8.35 | 88 | 10 | 5.11 | 25.5 | Removed liquid at 18 hr.; stored at room temp. |
| | | | 42 | 8.35 | 91 | 10 | | 36.2 | Fresh enzyme |
| 13 | 3 | 1 ml. of No. 10 | 18 | 5.6 | 78 | 200 | 5.30 | 8.2 | Heavy pellicle |
| | | | 20 | 5.65 | 80 | 20 | 5.11 | 12.3 | |
| | | | 20 | 5.65 | 84 | 10 | 5.15 | 20.2 | |
| | | | | 8.26 | 87 | 10 | 5.11 | 23.5 | Old enzyme stored at room temperature |
| | | | 41 | 8.26 | 92 | 10 | 5.25 | 31.7 | Fresh enzyme |
| 14 | 3 | 1 ml. of No. 10 | 89 | 8.7 | 108 | 10 | 5.28 | 76.8 | |
| 15 | Triple size batch No. 13 | 1.5 ml. of No. 10 | 41 | 5.7 | 90 | 10 | 5.19 | 25.4 | |
| | | | 65 | 5.6 | 97 | 10 | 5.08 | 22.1 | |
| Conc. 15 | - | -- | -- | -- | 112 | 10 | ≦5 | 17.3 | Vacuum concentrated enzyme liquids |
| Conc. 15 | - | -- | -- | -- | 119 | 5 | 5.22 | 20.5 | |
| Conc. 15 | - | -- | -- | -- | 120 | 2 | 5.17 | 29.6 | |
| Conc. 15 | - | -- | -- | -- | 131 | 2 | -- | 36.4 | Aged 14 days |
| Conc. 15 | - | -- | -- | -- | 134 | 2 | -- | 30.0 | |

TABLE VI

EXPERIMENTS WITH PURIFIED CULTURES OF THE BACILLUS X

| Enzyme No. | Substrate Comp. No. | Inoculant | 37° C. Incubation Time in hr. | pH of Enzyme | Determination of Enzyme Activity: Ex. No. | % Enzyme Added on Gum | pH | Activity at 30° | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 16 | 3 | 1 ml. of No. 13 | 20 | 5.55 | 95 | 10 | 5.09 | 24.7 | |
| | | | 26 | -- | 99 | 10 | 5.11 | 24.3 | |
| | | | 44 | 7.35 | 109 | 10 | 5.36 | 25.1 | |
| 17 | 3 | 2nd streak of No. 3 | 20 | 5.42 | 96 | 10 | 5.08 | 24.4 | |
| | | | 26 | -- | 100 | 10 | 5.11 | 23.2 | |
| | | | 50 | 8.05 | 110 | 10 | 5.42 | 31.4 | |
| 18 | 3 | 2nd streak subculture 1 | 20 | 5.40 | 98 | 10 | 5.17 | 25.4 | |
| | | | 26 | -- | 101 | 10 | 5.17 | 25.5 | |
| | | | 50 | 8.01 | 111 | 10 | 5.25 | 26.2 | |
| 21 | 3 | Same | 10 | 5.45 | 104 | 10 | 5.27 | 27.7 | All liquid |
| | | | 17 | 5.32 | 113 | 10 | -- | 25.6 | |
| | | | 34 | 7.27 | 115 | 10 | 5.17 | 22.2 | |
| 22 | 3 | 2nd streak culture 1 | 10 | 5.5 | 105 | 10 | 5.25 | 28.6 | Almost all liquid substrate |
| | | | 17 | 5.30 | 114 | 10 | 5.22 | 26.8 | |
| | | | 34 | 7.20 | 116 | 10 | 5.20 | 24.3 | |
| 23 | 3 | 1 ml. of No. 16 | 10 | 5.40 | 106 | 10 | 5.26 | 25.9 | All liquified |
| | | | 34 | 7.41 | 118 | 10 | 5.17 | 22.3 | |
| 24–28 | Triple size batches of | 1 ml. of second streak of subculture 1 | 45 | 7.33 | 121 | 10 | 5.28 | 24.5 | Centrifuged all and preserved with toluene in refrigerator called No. 26 |
| Called 26 | 3 | | *69 | -- | 128 | 10 | 5.30 | 26.5 | |
| | | | *285 | -- | 132 | 10 | -- | 34.4 | |
| | | | *309 | -- | 136 | 10 | -- | 30.2 | |
| 26 conc. | - | -- | - | -- | 129 | 2.0 | 5.32 | 23.6 | Vacuum concentrated |
| | | | | | 130 | 1.0 | 5.28 | 29.2 | |
| | | | | | 135 | 1.0 | ≦5.2 | 29.6 | Aged 10 days at 10° C. |

* Temperature is 10° C.

TABLE VII

EXPERIMENTS WITH SEVERAL APPARENT VARIANTS OF
BACILLUS X ON LOCUST BEAN GUM

| Enzyme No. | Substrate Comp. No. | Inoculant Type of Bacillus X | 37° C. Incubation Time in hr. | pH of Enzyme Liquid | Determination of Enzyme Activity: Ex. No. | % Enzyme Added on Gum | pH | Activity at 30° |
|---|---|---|---|---|---|---|---|---|
| 29 | 3 | Var. No. 2 | 24 | 6.48 | 123 | 10 | 5.12 | 21.2 |
| 30 | 3 | Var. No. 5 | 24 | 7.1 | 124 | 10 | 5.35 | 19.6 |
| 31 | 3 | Var. No. 4 | 24 | 6.0 | 125 | 10 | 5.22 | 20.6 |
| 33 | 3 | Var. No. 3 | 24 | 6.35 | 126 | 10 | 5.20 | 22.1 |
| 34 | 3 | Var. No. 1 | 24 | 6.5 | 127 | 10 | 5.20 | 19.2 |
| 37 | 3 | Var. No. 1 | 24 | 6.6 | 137 | 5 | 5.40 | 25.9 |
| | | | 45 | 8.35 | 142 | 5 | 5.20 | 50.8 |
| 38 | 3 | Var. No. 2 | 24 | 6.5 | 138 | 5 | 5.30 | 27.1 |
| | | | 45 | 8.4 | 143 | 5 | 5.15 | 55.3 |
| 39 | 3 | Var. No. 3 | 24 | 5.82 | 139 | 5 | 5.3 | 26.4 |
| | | | 45 | 8.1 | 144 | 5 | 5.25 | 49.5 |
| 40 | 3 | Var. No. 4 | 24 | 5.65 | 140 | 5 | 5.22 | — |
| | | | 45 | 8.05 | 145 | 5 | 5.12 | 25.4 |
| 41 | 3 | Var. No. 5 | 24 | 6.2 | 141 | 5 | 5.4 | 25.6 |
| | | | 45 | 8.4 | 146 | 5 | 5.25 | 51.2 |

TABLE VIII

BRIEF DESCRIPTION OF THE COLONIES OF VARIANTS OF BACILLUS X

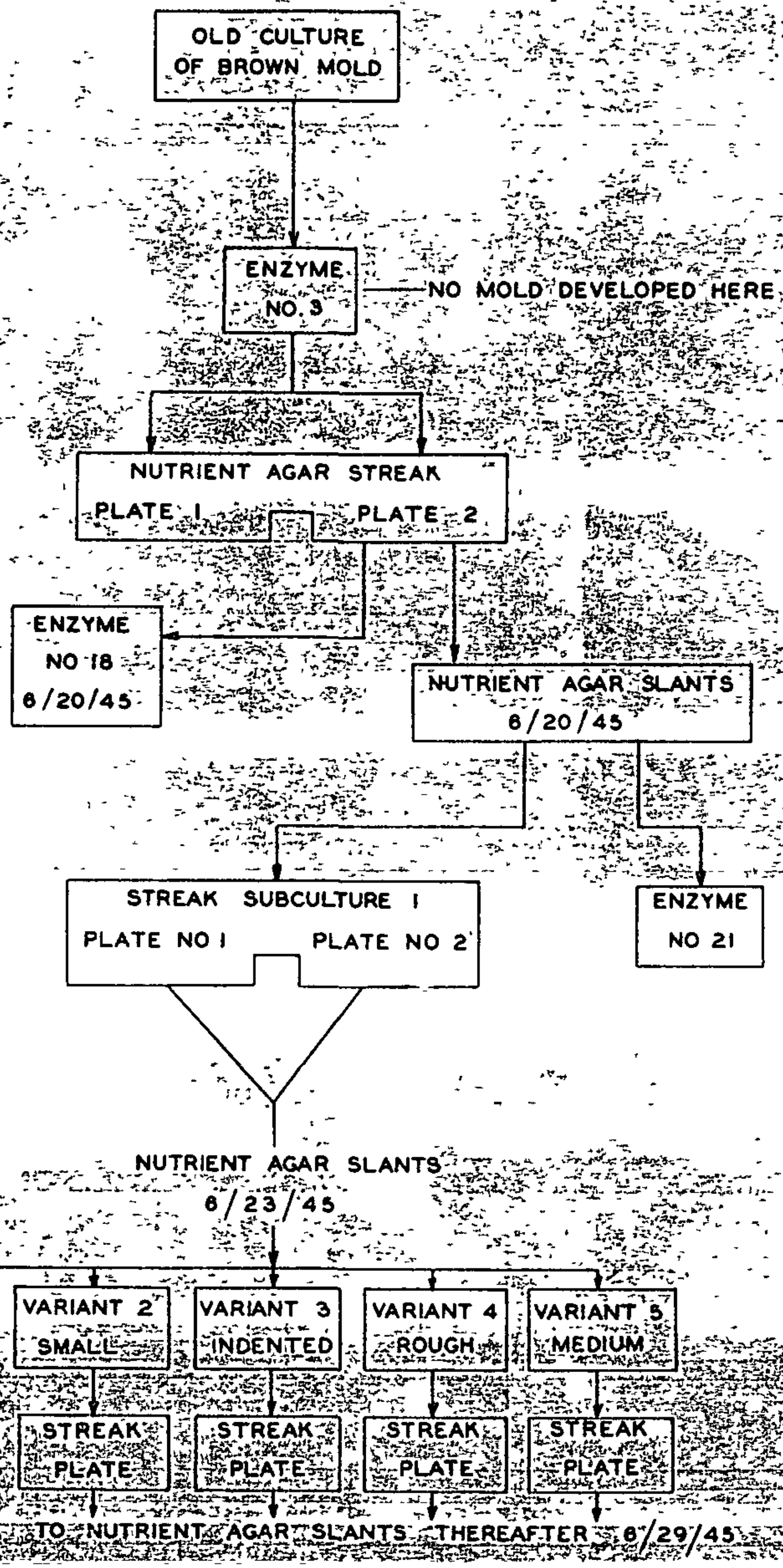
| Variant Number | Description of Growth |
|---|---|
| 1 | Circular colonies, smooth surface |
| 2 | Circular colonies, smaller in diameter than others |
| 3 | Colonies indented |
| 4 | Circular colonies, surface very rough and convoluted |
| 5 | Circular colonies, medium in size, smooth surface |

## VACUUM CONCENTRATION OF ENZYMES

During the work of isolating and testing the variants of Bacillus X, other experiments were made to concentrate and store the enzyme preparations with a minimum loss of activity. Previous attempts to concentrate the enzyme liquor by vacuum distillation of the water had not met with success because of persistent foaming. After several more failures, it was found possible to concentrate by this method providing the liquor was first saturated with toluene and the distillation begun at 20-25° C. After the main fraction of toluene had distilled off, the temperature could be slowly increased to 35-38° C.

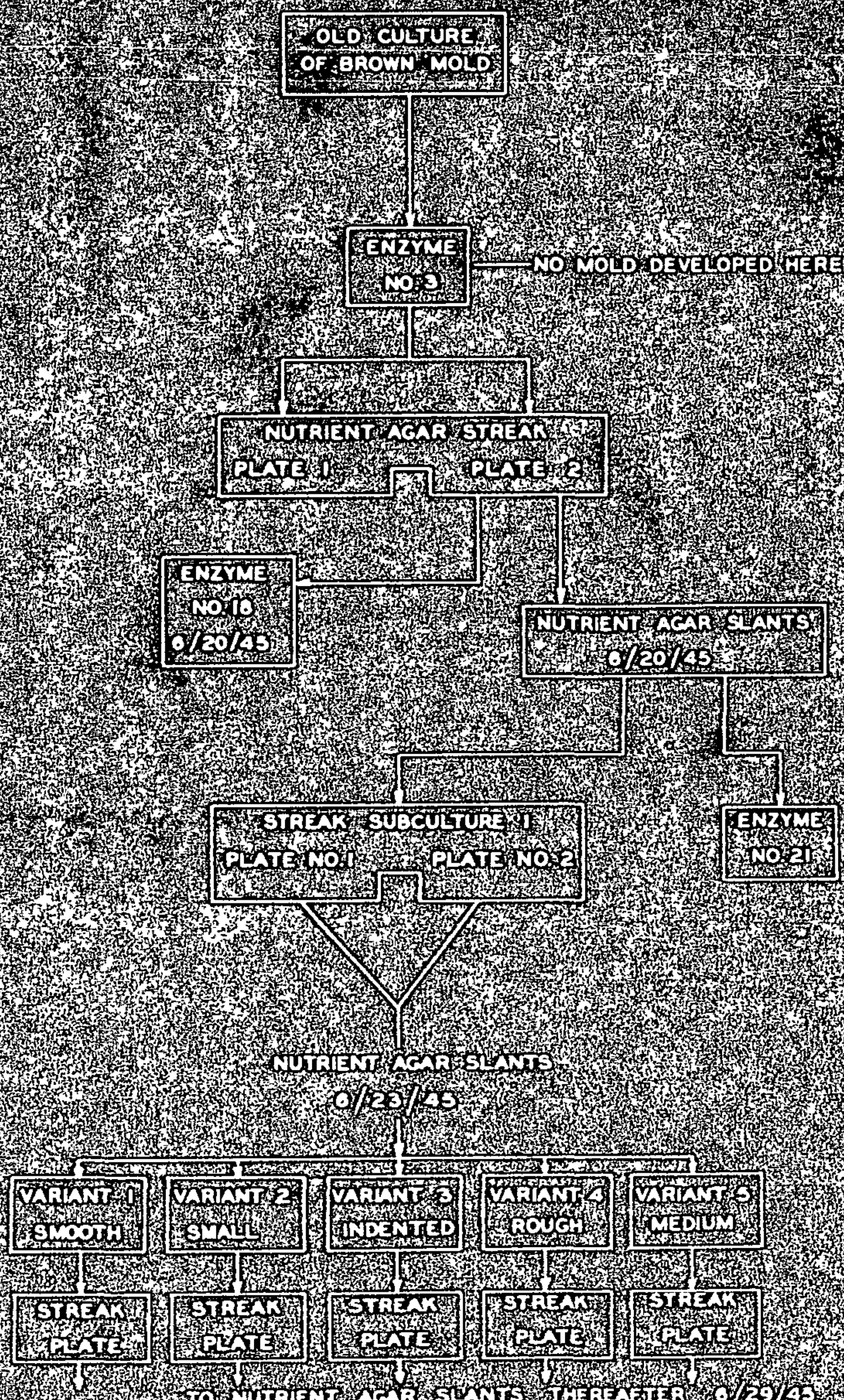
## CHART I

FLOW CHART SHOWING SOURCE OF BACILLUS X AND THE SEVERAL VARIANTS



## CHART I

FLOW CHART SHOWING SOURCE OF BACILLUS X AND THE SEVERAL VARIANTS



and the pressure held at about 20 mm. This gave a fairly rapid method of concentrating the enzyme anywhere from 5 to 18 times the original potency. During most of the concentration experiments, carbon dioxide was bubbled through the mixture instead of air. It has not been ascertained whether or not this is an improvement or not.

A series of four triple-size batches of enzyme cultures were made and temporarily called Enzymes 24 to 28. These flasks were inoculated with a subculture of Bacillus X (see Table VI), and after measuring the potency at 45 hours incubation time, the cultures were combined, filtered, centrifuged, and vacuum concentrated. This mixture was called Enzyme No. 26 and showed a very high potency at a concentration of 1% on the weight of mucilage used for determining the activity. It was believed to be sufficiently potent to be used for converting mucilage for tubsizing experiments. In order to use the enzyme under nearly optimum conditions, several preliminary experiments were made to determine the effects of pH, temperature and time on the activity. These experiments are given in Tables IX and X and indicate that the optimum pH at 30° C. is about 6.6 and that the enzyme does not lose activity at a prohibitive rate when held at 70° C. and pH of 5.95. The experimental tubsize conversions will be dealt with in a later section of this report.

TABLE IX

THE EFFECT OF AGING CONCENTRATED ENZYME NO. 26 AT VARIOUS TEMPERATURES AND pH = 5.95

(Locust Bean Gum Substrate)

| Ex. No. | Temperature of aging, °C. | Time Held at Temp., min. | Activity at 30° C. |
|---|---|---|---|
| 162 | 50 | 10 | 35.8 |
| | | 20 | 35.9 |
| | | 30 | 52.6* |
| | | 60 | 38.1 |
| 163 | 60 | 10 | 40.9 |
| | | 20 | 41.7 |
| | | 30 | 42.9 |
| | | 60 | 47.6 |
| 164 | 70 | 10 | 61.3 |
| | | 20 | 71.8 |
| | | 30 | 67.8 |
| | | 60 | 78.2 |

* Possibly due to unclean viscometer.

## TABLE X

### EXPERIMENTS WITH CONCENTRATED NO. 26 ENZYME ON LOCUST BEAN GUM AND GUAR MUCILAGES

| Ex. No. | % Enzyme Added on Gum | Buffer Kind | Buffer Amt. ml. | pH | Temp. °C. | Activity |
|---|---|---|---|---|---|---|
| 147 | 5 | HAc | 2.8 | 3.91 | 30 | 82.7 |
| 148 | 5 | $NaHCO_3$ | 0.05 | 6.31 | 30 | 16.3 |
| 149 | 2 | HAc | 2.8 | 3.87 | 30 | 94.8 |
| 150 | 2 | HAc | 0.15 | 5.12 | 30 | 22.4 |
| 151 | 2 | $NaHCO_3$ | 0.05 | 6.39 | 30 | 20.6 |
| 152 | 2 | $NaHCO_3$ | 0.65 | 7.31 | 30 | 20.9 |
| 153 | 2 | $NaHCO_3$ | 5.0 | 8.3 | 30 | 22.8 |
| 154 | 2 | NaOH | 2.6 | 9.1 | 30 | 28.6 |
| 155 | 2 | HAc | 0.15 | 5.25 | 40 | 23.7 |
| 156 | 2 | $NaHCO_3$ | 0.05 | 6.48 | 40 | 21.1 |
| 157 | 2 | $NaHCO_3$ | 0.65 | 7.3 | 40 | 21.6 |
| 158 | 2 | $NaHCO_3$ | 5.0 | 8.42 | 40 | 23.7 |
| 159 | 2 | NaOH | 2.6 | 9.08 | 40 | 25.6 |
| 160 | 2 | HAc | 0.7 | 4.38 | 30 | 71.5 |
| 161 | 2 | NaOH | 5.0 | 9.9 | 30 | 25.1 |
| | | Guar Mucilage | | | | |
| 165 | 2 | HAc | 0.15 | 5.38 | 30 | 57.8 |
| 166 | 2 | NaOH | 5.0 | 9.84 | 30 | 72.8 |
| 167 | 2 | $NaHCO_3$ | 5.0 | 8.27 | 30 | 50.9 |
| 168 | 2 | HAc | 0.7 | 4.58 | 30 | 99.6 |
| 169 | 2 | $NaHCO_3$ | 0.35 | 7.1 | 30 | 48.9 |

Attempts to Grow Bacillus X on Guar Mucilage

Since ultimate industrial application of a suitable enzyme would be made principally upon guar mucilage, several experiments with No. 26 were made on guar G4-5; See Table X. The very disappointing discovery was made that the potency of concentrated No. 26 enzyme on guar mucilage was very much lower than when locust bean gum was used. Furthermore, attempts to grow Bacillus X on guar mucilage met with failure insofar as enzyme production is concerned. The organism exists on guar substrate but the enzyme produced seems to attack only a relatively small proportion of the guar molecule. The viscosity falls very slowly for a time and then seems to remain fairly constant. Attempts to improve growth by changes in purity of mucilage (eliminating possible poisons), nutrient salts, peptone content, and concentration of mucilage has not thus far improved the enzyme potency. Experiments have also been made to condition the organism toward growing on guar by using mixtures of guar and locust bean gum in various ratios. Thus far, after 10 generations on a substrate composed of 66.6% guar and 33.3% locust bean gum plus peptone and nutrient salts, no increase in enzymatic activity could be noted.

Experiments with Several Known Bacteria

Several known organisms were used for inoculating both guar and locust bean gum substrates. Among those used were Bacillus subtilis,

TABLE XI

EXPERIMENTS ON GROWTH OF BACILLUS X ON VARIOUS GUAR SUBSTRATES

| Enzyme No. | Substrate Comp. No. | Inoculant | 37° C. Incubation Time in hr. | pH of Enzyme | Determination of Enzyme Activity: Ex. No. | % Enzyme Added on Gum | pH | Activity on G4-5 | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 43 | Triple batch of No. 8 Guar | Variant No. 1 | 18 | 7.12 | - | — | — | — | Substrate liquified but remained viscous |
| | | | 66 | 8.1 | 170 | 10 | 5.47 | 99.9 | Same |
| 50 | 8 guar | Variant No. 2 | 16 | — | 171 | 10 | 5.11 | 79.8 | Same |
| | | | 30 | 8.36 | 175 | 10 | 5.58 | 78.3 | Same |
| | | | 54 | — | 185 | 10 | 5.34 | 89.1 | |
| 51 | 9 guar | Variant No. 2 | 16 | — | 172 | 10 | 5.18 | 83.8 | |
| | | | 30 | 7.94 | 176 | 10 | 5.48 | 91.8 | Same |
| 52 | 8 guar | Culture of No. 3 Enzyme | 16 | — | 174 | 10 | 5.19 | 87.9 | |
| | | | 54 | 8.47 | 177 | 10 | 5.56 | 99.9 | Same |
| 53 | 3 Purified LBG | Variant No. 1 | 36 | — | 183 | 10 | 5.28 | 43.7 | |
| 55 | 10 guar | Culture of No. 50 Enzyme | 16 | 7.89 | 179 | 10 | 5.55 | 88.0 | |
| 56 | 11 guar | Variant No. 1 | 16 | 7.29 | 180 | 10 | 5.50 | 80.8 | Added lactose to substrate |
| | | | 30 | — | 186 | 10 | 5.32 | 63.6 | |
| 57 | 12 LBG and G4-5 | Variant No. 1 | 16 | 6.5 | 178 | 10 | 5.48 | 59.1 | |
| | | | 30 | — | 184 | 10 | 5.28 | 51.2 | |
| 58 | 13 G4-2 | Variant No. 1 | 16 | 7.89 | 181 | 10 | — | 90.4 | Substrate viscous |
| 59 | 14 dilute guar | Variant No. 1 | *15 | 7.35 | 189 | 10 | 5.47 | 97.0 | |
| | | | *22 | 7.8 | 192 | 10 | 5.55 | 92.2 | |
| | | | *87 | 7.77 | 195 | 10 | 5.47 | 95.7 | |
| 60 | Same as above, No. 14 except LBG used | Variant No. 1 | *15 | 6.73 | 190 | 10 | 5.47 | 88.7 | Locust bean gum activity was 53.8 |
| | | | *22 | 7.5 | 193 | 10 | 5.52 | 79.0 | |
| | | | *87 | 8.38 | 196 | 10 | 5.5 | 91.5 | |

* Temperature is 30° C.

TABLE XII

ENZYMES FROM SEVERAL KNOWN ORGANISMS GROWN ON LOCUST BEAN GUMS AND GUAR MUCILAGES

| Enzyme No. | Substrate Comp. No. | Inoculant | 37° C. Incubation Time in hr. | pH of Enzyme | Determination of Enzyme Activity: Ex. No. | % Enzyme Added on Gum | pH | Activity at 30° C. | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 19 | 3 | Bacillus subtilis | 26 | Partially liquid | 102 | 10 | 5.20 | 88.8 LBG | |
| | | | 50 | 5.57 | 107 | 10 | 5.13 | 88.7 LBG | |
| 20 | 3 | Bacillus mycoides | 20 days | No liquid | -- | -- | -- | -- | |
| 35 | 3 | Bacillus macerans | 12 | -- | -- | -- | -- | -- | Substrate was lumpy |
| 36 | 3 | Bacillus mesentericus | 10 days | -- | -- | -- | -- | -- | No liquifaction |
| 63 | 10 guar | Bacillus mycoides | | | | | | | No liquifaction |
| 64 | 10 guar | Bacillus mesentericus | | | | | | | No liquifaction |
| 65 | 10 guar | Bacillus subtilis | 44 | 8.67 | 201 | 10 | 5.32 | 91.8 guar | Viscous fluid |
| 69 | 10 guar | Bacillus macerans | 22 | 5.63 | 203 | 10 | 5.20 | 94.1 guar | All liquified; not viscous |
| | | | 28 | 5.5 | 206 | 10 | 5.25 | 99.9 guar | |
| | | | 46 | 5.69 | 209 | 10 | 5.44 | 64.8 guar | |
| 90 | 17 guar | Aspergillus oryzae | *96 | -- | 242 | 10 | 5.47 | 72.6 guar | |
| | | | *144 | -- | 253 | 10 | -- | 87.2 guar | |

* Temperature is 30° C.

Bacillus mycoides, Bacillus macerans, and Bacillus mesentericus. Bacillus subtilis and Bacillus macerans partly liquified locust bean gum and guar mucilages but the enzyme potency was poor. The other organisms grew on the substrates but failed to liquify them.

A strain of Aspergillus oryzae known to be a particularly potent producer of amylase enzymes when grown on starch was used for inoculating a guar substrate. Liquifaction occurred but the enzyme potency was of a low order.

Experiments with Soil Infusions

Since soils are known to contain so many varieties of bacteria, several attempts were made to isolate species which might completely liquify guar mucilage. The soil samples were simply mixed with water in a test tube and one ml. samples of each were pipetted into flasks of sterile guar, peptone, salt substrate. The cultures were liquified but enzyme activity was nil after 72 hours growth. Transfers from the cultures were made to fresh substrate which were also liquified but no enzyme activity was evident. See Table XIII. Nutriet agar streak plate cultures were made of the latter growths, and 3 distinctive colonies of organisms were separated. None of these organisms liquified guar mucilage although good growth was evident.

Experiments with Mold Enzymes

When considerable difficulties were encountered in the use of the bacterial enzyme on guar mucilage, it seemed expedient to

TABLE XIII

SOIL INFUSIONS GROWN ON GUAR MUCILAGE

| Enzyme No. | Substrate Comp. No. | Inoculant | 37° C. Incubation Time in hr. | pH of Enzyme | Determination of Enzyme Activity: Ex. No. | % Enzyme Added on Gum | pH | Activity on G4-5 | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 71 | 10 guar | Soil Infusion No. 1 | 72 | 4.95 | 233 | 10.0 | 5.50 | 99.9 | |
| 72 | 10 | Soil Infusion No. 2 | 72 | 4.75 | 234 | 10.0 | 5.50 | 99.9 | |
| 73 | 10 | Soil Infusion No. 3 | 72 | 5.37 | 235 | 10.0 | 5.51 | 99.9 | |
| 74 | 10 | Soil Infusion No. 4 | 72 | 4.9 | 236 | 10.0 | 5.47 | 97.8 | |
| 75 | 10 | 1 ml. of Enzyme Culture No. 71 | 19 | 5.09 | 237 | 10.0 | 5.48 | 99.9 | |
| 76 | 10 | 1 ml. of Enzyme Culture No. 72 | 19 | 6.13 | 238 | 10.0 | 5.48 | 99.9 | |
| 77 | 10 | 1 ml. of Enzyme Culture No. 73 | 19 | 5.70 | 239 | 10.0 | 5.48 | 99.9 | |
| 78 | 10 | 1 ml. of Enzyme Culture No. 74 | 19 | 5.71 | 240 | 10.0 | 5.51 | 99.9 | |
| 81-88 | 17 | Pure cultures isolated from plate cultures of No. 75 to 78; no enzyme produced. | | | | | | | |

Note: Sources of soil--No. 1 Garden at home--527 E. South River Street
No. 2 Newly-plowed ground north of Institute paper house
No. 3 Soil from ground south of paper house
No. 4 Clay soil from trench dug to manhole at northwest corner of 1942 Institute building

continue the work with the Brown mold enzymes while further attempts were made to find a bacterium suitable for guar. The possible advantages and disadvantages of mold and bacterial enzymes will be discussed in a later section.

It has been found by adjusting the substrate with peptone nutrient salts and buffer, purifying the culture of Brown mold, and growing for the optimum length of time, that the enzyme activity of the liquor could be increased markedly. In fact, the activity on guar and locust bean gum was greater than the Bacillus X enzyme on locust bean gum. These experiments are summarized in Table XIV.

A large batch of 4 flasks of Enzyme No. 70 was made and concentrated under vacuum. Determination of the optimum pH at 30° C. gave a value of approximately 3.8 to 4.0. This figure should not be accepted as rigid since several buffers were used for adjusting pH values and it is not known whether or not the activity is markedly affected by the kind of buffer. Concentrated No. 70 enzyme was used for several experimental tubsize conversions of guar G4-5. These are given in a later section.

The activity of No. 70 and its possible application as a converting agent for guar led to the production of larger quantities of mold enzyme. The intention was to accumulate a sufficient quantity for several mill trials if the proper manner of using the enzymes could be determined. These experiments are given in Table XVI. The first

TABLE XIV

EXPERIMENTS WITH MOLD ENZYMES

| Enzyme No. | Substrate Comp. No. | Inoculant | 37° C. Incubation Time in hr. | pH of Enzyme | Determination of Enzyme Activity: Ex. No. | % Enzyme Added on Gum | pH | Activity on 04-5 | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 54 | 15 | Pure brown mold | 120 | 7.93 | 197 | 10 | 5.48 | 26.2 | |
| 62 | 15 | Spores of Enzyme No. 54 | * 46 | 7.41 | 200 | 10 | 5.20 | 77.2 | Made three flasks of identical composition |
| | | | * 52 | 7.35 | 204 | 10 | 5.18 | 82.4 | |
| | | | * 70 | 6.42 | 207 | 10 | 5.48 | 20.8 | |
| | | | *120 | 6.0 | 218 | 10 | 5.42 | 15.7 | |
| Concentrated 62 | | | | | 211 | 1.0 | 5.48 | 12.6 | Vacuum concentrated 10 times |
| | | | | | 214 | 1.0 | 5.44 | 14.8 | Two days old |
| | | | | | 215 | 1.0 | 4.55 | 14.3 | |
| | | | | | 216 | 1.0 | 8.77 | 30.4 | |
| | | | | | 217 | 1.0 | 5.41 | 14.0 | Four days old |
| | | | | | 221 | 1.0 | 5.11 | 12.2 | Activity on LBG |
| Concentrated 26 | | | | | 222 | 1.0 | 5.09 | 21.9 | Activity on LBG |
| 66 | 10 | 1 ml. of mold of Enzyme No. 54 | * 53 | 2.39 | 210 | 10 | 5.45 | 39.8 | |
| | | | * 72 | 2.16 | 213 | 10 | 5.38 | 40.7 | |
| | | | *120 | 4.67 | 219 | 10 | 5.41 | 21.6 | |
| 67 | 10 | Mold from No. 54 and Variant No. 3 | * 22 | 8.22 | 202 | 10 | 5.19 | 81.9 | No mold growth; pellicle formed; fluid viscous |
| | | | * 28 | 8.37 | 205 | 10 | 5.30 | 92.1 | |
| 68 | 10 | Mold from No. 54 and after | * 46 | 4.01 | 208 | 10 | 5.45 | 67.6 | Both mold and bacillus appeared to be growing |
| | | 29 hours added Variant No. 3 | * 52 | 3.73 | 212 | 10 | 5.48 | 46.6 | |

*Temperature 30° C.

of the large batches, (20 flasks-triple size batches) No. 89, became contaminated with another mold type organism and was not representative of the type previously made on a small scale. However, this enzyme was quite potent. It possessed an alkaline pH and was intensely black in color whereas other products were brown. A further evaluation of this enzyme will be made.

Purification of the mold culture was again necessary, and following this, batches of 10 flasks of No. 97, No. 97-2, and No. 97-3 were grown over several weeks time. These were concentrated under vacuum. The only noticeable difference between the enzyme liquors of the three batches was in the final pH. No. 97 had a pH of 3.5 whereas No. 97-2 and 97-3 were both 6.45. The significance of the pH values has not been fully determined as yet but experiments are in progress on this factor.

TABLE XV

EXPERIMENTS WITH MOLD ENZYME NO. 70

| Enzyme No. | Substrate Comp. No. | Inoculant | 30° C. Incubation Time in hr. | pH of Enzyme | Determination of Enzyme Activity: Ex. No. | % Enzyme Added on Gum | pH | Activity on G4-5 | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 70 | 16 | Culture from Enzyme No. 62 | 66 | 6.0 | 220 | 10.0 | 5.40 | 36.4 | Four flasks made up |
| | | | 118 | 3.34 | 223 | 10.0 | 5.40 | 17.7 | |
| | | | | 3.31 | 224 | 10.0 | 5.40 | 17.8 | Mixed four flasks |
| Concentrated 70 | | — | — | — | 225 | 0.5 | 5.38 | 13.8 | Vacuum concentrated |
| | | | | | 226 | 0.5 | 3.60 | 13.0 | HAc 7.5 ml. buffer |
| | | | | | 227 | 0.5 | 7.40 | 18.1 | $NaHCO_3$ 0.65 ml. |
| | | | | | 228 | 0.5 | 8.57 | 30.0 | NaOH " 2.6 ml. |
| | | | | | 229 | 0.5 | 5.31 | 17.5 | HAc buffer |
| | | | | | 230 | 0.5 | 1.65 | 21.6 | HCl buffer |
| | | | | | 231 | 0.5 | 2.44 | 17.8 | Oxalic acid buffer |
| Concentrated 70 | | 31 days old; stored at 10° C. | | | 232 | 0.5 | 5.47 | 21.1 | |
| | | | — | — | 265 | 0.05 | 5.4 | 50.6 | |
| | | | | | 266 | 0.10 | — | 39.6 | |
| | | | | | 267 | 0.20 | — | 28.3 | |
| | | | | | 268 | 0.50 | — | 17.3 | |
| | | | Low viscosity mucilage used | | 256 | 0.50 | 5.40 | 42.3 | 0.11% in boric acid |
| | | | | | 257 | 0.50 | 5.35 | 42.2 | 0.22% in boric acid |
| | | | | | 258 | 0.50 | — | 42.3 | No boric acid |
| | | | | | 261 | 0.50 | 5.26 | 17.4 | Higher blank used |

TABLE XVI
GROWTH OF SEVERAL LARGE BATCHES OF MOLD ENZYMES

| Enzyme No. | Substrate Comp. No. | Inoculant | 30° C. Incubation Time in hr. | pH of Enzyme | Determination of Enzyme Activity: Ex. No. | % Enzyme Added on Gum | pH | Activity on G4-5 | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 89 | 18 guar | Mold from No. 70 | 87 | 6.9 | — | 10 | 6.15 | 49.3 | Made 20 flasks of this composition; they appeared to be contaminated at 116 hr. |
| | | | 116 | 6.78 | — | 10 | 5.38 | 41.0 | |
| | | | 140 | 6.97 | — | 10 | 5.4 | 40.2 | |
| | | | 164 | 7.5 | — | 10 | 5.4 | 36.4 | |
| | | | 188 | — | — | 10 | — | 36.4 | |
| | | | 228 | 7.8 | 241 | 10 | — | 36.8 | |
| | | | 252 | 7.73 | 250 | 10 | 5.4 | 37.8 | |
| Concentrated 89 | | | — | — | 264 | 0.5 | — | 11.9 | |
| 91 | 19 guar | Mold from No. 70 | 108 | 7.08 | 243 | 10 | 5.45 | 39.9 | |
| | | | 132 | 4.08 | 251 | 10 | 5.39 | 38.1 | |
| 92 | Triple batch of 10 | Mold from No. 70 | 108 | 7.15 | 244 | 10 | 5.44 | 39.7 | Contaminated |
| | | | 132 | 3.58 | 252 | 10 | 5.37 | 37.3 | Contaminated |
| 94 | 17 | Guar seeds | * 40 | — | 254 | 10 | — | 55.4 | |
| 96 | 17 | 1/4 ml. of No. 94 | * 16 | — | 255 | 10 | — | 64.0 | |
| 97 | 20 | Pure culture Brown mold No. 95 | 93 | 6.85 | 259 | 10 | 5.29 | 20.1 | |
| | | | 117 | — | 262 | 10 | — | 17.9 | |
| | | | 142 | 3.6 | 269 | 10 | — | 15.6 | |
| Concentrated 97 | | | — | 3.4 | 272 | 0.5 | — | 15.7 | Vacuum concentrated about 17.6 times |
| 97-2 | 20 | Mold No. 95 | 144 | 6.45 | 274 | 10.0 | — | 14.2 | |
| Concentrated 97-2 | | | — | 6.28 | 275 | 0.5 | — | 17.7 | Vacuum concentrated about 16.7 times |
| 97-3 | 20 | Mold No. 95 | 144 | 6.45 | 276 | 10.0 | — | 17.9 | |
| Concentrated 97-3 | | | — | — | Activity not determined | | | | Vacuum concentrated about 18 times |

*Temperature is 37° C.

EXPERIMENTAL ENZYME CONVERSIONS OF MUCILAGES FOR TUBSIZING PURPOSES

Conversion of Locust Bean Gum with
Bacterial Enzyme No. 26

Procedure. Three hundred ml. of water were added to a 600 ml. tared beaker and 0.15 ml. of No. 26 concentrated enzyme added. Fifteen grams of locust bean gum were added with vigorous stirring and steam injected until the temperature attained 50° C. After seven minutes an additional 0.15 ml. (total of 2% on gum) of enzyme was added and the temperature maintained at 45-47° C. for the desired length of time. (A rough check on the rate of conversion was made by measuring the viscosity in a 10 ml. pipette.) The temperature was then raised to 90° C. and held at 85-90° C. for 5 minutes to inactivate the enzyme. The concentration of mucilage was adjusted if desired and a tub-size run made with the small copper trough at about 55° C.

The action of No. 26 enzyme was tried on guar mucilage G4-5 but the action was very slow even at an enzyme concentration of 5% on the mucilage. After several attempts to improve the action at various pH, it became evident that No. 26 would not be suitable for converting guar mucilage.

Conversion of Guar Mucilage G4-5 with Concentrated Mold Enzymes

The property of guar mucilages to form doughballs when placed in water was somewhat troublesome at first because it is augmented by high concentration. Usually doughballing can be minimized or eliminated by adding the mucilage to dilute borax solution and later acidifying to a pH of 5 to 6.5. The literature indicated that boric acid is generally detrimental to enzyme activity and several attempts were made to disperse the mucilage by other means. Humidification and using cold water at 0° C. helped prevent doughball formation to a considerable extent but were still not satisfactory. It was decided that the boric acid tolerance of the enzyme should be determined to find out how much borax, if any, could be used. Surprisingly enough, concentrations of boric acid equal to twice the amount normally present in a borax cook did not appear to be detrimental to the enzyme action. This discovery eliminated the bothersome doughball formation.

Procedure. Fifteen grams of G4-5 were slurried in 270 ml. of 0.25% borax solution to which 2.8 g. of anhydrous (C. P.) calcium chloride were added. The temperature was raised to 65° C. by direct steam injection, and 13 ml. of 0.31 N HCl were added. The beaker was then immersed in a water bath held at 70° C. and 0.15 ml. of the concentrated enzyme was added. The mixture was held at 65° C. for the desired length of time and then steam was rapidly injected to raise the temperature to 92° C. where it was held for 5 minutes. The concentration was adjusted to the desired value and the mucilage used as a tubsize at 50-60° C. The relative viscosity was measured at 1% and 30° C. in an Ostwald viscometer and the pH determined with the glass electrode.

TABLE XVII

TUBSIZE CHARACTERISTICS OF SEVERAL ENZYME CONVERTED MUCILAGES (100% RAG STOCK)

| Institute File No. | Conversion Number * | Enzyme Used: Kind | Enzyme Used: % on Gum | Conversion Conditions: pH | Conversion Conditions: Temp., °C. | Conversion Conditions: Time, Min. | Tubsizing Conditions: Relative Viscosity at 1%, 30° C. | Tubsizing Conditions: Concentration | Tubsizing Conditions: Temp., °C. | Basis Weight, lb. 17x22/500 | Bursting Strength: Points | Bursting Strength: Points per 100 lb. | Per Cent Increase in Burst | M.I.T. Fold: In. | M.I.T. Fold: Across | Elmendorf Tear, g./sheet: In. | Elmendorf Tear, g./sheet: Across |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | **Locust Bean Gum** | | | | | | | | | |
| | | | | | | | | | | 19.6 | 31.3 | 160 | | 153 | 60 | 92 | 106 |
| | T155-631 | 26 | 2*** | 5.4 | 45-47 | 40 | | 4.0 | 55 | 19.5 | 42.1 | 216 | 35.0 | | | | |
| | T159-631 | 26 | 2*** | | 50-52 | 55 | 5.2 (2%) | 4.0 | 55 | 18.8 | 39.0 | 207 | 29.4 | | | | |
| | | | | | | | | 2.0 | 55 | 19.1 | 39.4 | 206 | 28.8 | | | | |
| | | | | | | | | **Guar Mucilage G4-5** | | | | | | | | | |
| 120367 | T48-1-654 | 70 | 1*** | 4.98 | 45-48 | 30 | | 4.45 | 53-60 | 19.3 | 36.4 | 189 | 18.1 | 438 | 86 | 92 | 102 |
| 120368 | T48-2-654 | 70 | 1*** | 5.02 | 47-48 | 30 | | 4.27 | 53-60 | 19.5 | 37.6 | 193 | 20.6 | 332 | 75 | 92 | 105 |
| 120790 | T64-654** | 70 | 1*** | 5.33 | 63-66 | 50 | | 4.0 | 55 | 19.5 | 39.6 | 203 | 26.9 | 360 | 78 | 90 | 103 |
| 120791 | T65-654 | 70 | 1 | 4.85 | 65 | 40 | 2.36 | 4.0 | 56 | 19.6 | 39.4 | 201 | 25.6 | 485 | 93 | 90 | 100 |
| 120792 | T67-654 | 70 | 1 | 4.82 | 65 | 25 | 2.59 | 4.0 | 57 | 19.7 | 40.2 | 204 | 27.5 | 430 | 109 | 91 | 96 |
| 120793 | T67-654 | | | | | | | 2.0 | 53 | 19.5 | 38.8 | 199 | 24.4 | 340 | 72 | 91 | 104 |
| 120794 | T68-654 | 70 | 1 | | 65 | 20 | 3.14 | 4.0 | 52 | 19.6 | 40.2 | 205 | 28.2 | 380 | 99 | 89 | 101 |
| 120795 | T68-654 | | | | | | | 2.0 | 56 | 19.0 | 39.1 | 206 | 28.8 | 309 | 87 | 93 | 106 |
| 120796 | T69-654 | 70 | 1 | 6.18 | 65 | 45 | 2.05 | 4.0 | 55 | 19.8 | 39.4 | 199 | 24.4 | 377 | 88 | 89 | 104 |
| 120797 | T69-654 | | | | | | | 2.0 | 57 | 19.0 | 39.1 | 206 | 28.8 | 337 | 99 | 95 | 108 |
| 120798 | T70-654 | 70 | 1 | 5.0 | 75 | 20 | 2.73 | 4.0 | 60 | 21.1 | 40.8 | 193 | 20.6 | 377 | 99 | 91 | 105 |
| 120799 | T70-654 | | | | | | | 2.0 | 57 | 19.0 | 37.8 | 199 | 24.4 | 348 | 75 | 98 | 99 |
| 120800 | T73-654 | 70 | 1 | 6.2 | 75 | 85 | 2.91 | 4.0 | 59 | 20.0 | 42.2 | 211 | 31.9 | 407 | 84 | 89 | 99 |
| 120801 | T73-654 | | | | | | | 2.0 | 59 | 18.9 | 38.7 | 205 | 28.2 | 315 | 95 | 92 | 101 |
| 120869 | | | | | | | | | | 19.6 | 31.3 | 160 | | 153 | 60 | 92 | 106 |
| 120867 | T84-654 | 70 | 0.53 | 4.8 | 75 | 40 | 3.4 | 4.0 | 56 | 21.2 | 40.5 | 191 | 19.4 | 385 | 97 | 91 | 102 |
| 120868 | T84-654 | | | | | | | 2.0 | 57 | 19.7 | 38.2 | 194 | 21.2 | 199 | 62 | 95 | 108 |
| 120937 | Tera mucilage* T103-654 | 70 | 1.0 | 4.05 | 62 | 6 | 2.45 | 4.0 | 58 | 19.9 | 40.8 | 205 | 28.1 | 391 | 78 | 91 | 97 |
| 120938 | Tera mucilage T103-654 | | | | | | | 2.0 | 55 | 19.9 | 37.7 | 189 | 18.1 | 348 | 74 | 96 | 107 |
| 121055 | T114-654 | 97-2 | 1.0*** | 5.38 | 65 | 15 | 3.77 | 4.0 | 58 | 19.9 | 40.2 | 202 | 26.2 | 334 | 79 | 92 | 105 |
| 121056 | T114-654 | | | | | | | 2.0 | 57 | 19.5 | 38.4 | 196 | 22.5 | 287 | 75 | 97 | 103 |
| 121057 | T117-654 | 97-2 | 1.0 | 4.71 | 65 | 20 | 2.68 | 4.0 | 58 | 20.0 | 42.0 | 210 | 31.2 | 357 | 67 | 89 | 108 |
| 121058 | T117-654 | | | | | | | 2.0 | 58 | 19.9 | 38.5 | 193 | 20.6 | 251 | 67 | 91 | 109 |
| 121439 | T131-654 | 97-2 | 5.0 | 7.1 | 65 | 85 | 4.09 | 4.0 | 56 | 19.9 | 40.4 | 203 | 26.9 | | 113 | 92 | 97 |
| 121440 | T131-654 | | | | | | | | 56 | 19.8 | 37.6 | 190 | 18.9 | 331 | 81 | 94 | 103 |

* Page and notebook number where conversion data may be found

**All succeeding conversions made in presence of boric acid

***No $CaCl_2$ added

Starch Tubsizes for Comparison Purposes

In Table XVIII will be found some data on a hypochlorite converted guar mucilage and a commercial hypochlorite treated corn starch, Superfilm No. 4. These products were cooked in the normal manner for these materials and used as tubsizes at the temperatures designated in the table.

DISCUSSION

The most desirable type of enzyme for converting a polysaccharide to a suitable adhesive for tubsizing and coating is one which will attack the macromolecule in such a way as to give a suitable reduction in viscosity with a minimum production of sugars. Stated differently, the final converted product should have the highest possible average molecular weight at the desired viscosity in order to possess the maximum adhesive strength. In the foregoing experiments of this report, enzymes have been developed which are entirely adequate from the standpoints of potency and speed of conversion to a desired viscosity. The bacterial enzyme from Bacillus X was not fully evaluated on locust bean gum but preliminary tubsize data were encouraging. The Brown mold enzyme was more fully evaluated on guar G4-5 as a tubsize but, the results were not outstanding. The reasons for this may be several and these are being investigated.

It might be said that the problem of mannogalactan enzyme conversion now stands at a stage of development comparable to starch enzyme conversion 20 years ago--the enzymes were isolated and produced

# TABLE XVIII

TUBSIZE CHARACTERISTICS OF CHLORINATED MANNOGALACTAN AND SUPERFILM NO. 4 STARCH FOR COMPARISON WITH THE ENZYME CONVERTED MUCILAGE

| Institute File No. | Type of Adhesive | Tubsizing Conditions: Relative Viscosity at 1%, 30° C. | Tubsizing Conditions: Concentration, % | Tubsizing Conditions: Temp., °C. | Basis Weight, lb. 17x22/500 | Bursting Strength: Points | Bursting Strength: Points per 100 lb. | Per Cent Increase in Burst | M.I.T. Fold: In. | M.I.T. Fold: Across | Elmendorf Tear g./sheet: In. | Elmendorf Tear g./sheet: Across |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Chlorinated G44-G151-573 | | | | | | | |
| 114192 | Guar mucilage | 3.7 | 1.0 | 50 | 18.7 | 36.5 | 195 | 21.9 | 264 | 75 | 87 | 97 |
| 114191 | | | 2.0 | 50 | 18.9 | 40.4 | 214 | 33.8 | 357 | 102 | 84 | 94 |
| 114190 | | | 3.5 | 50 | 19.7 | 43.3 | 227 | 41.9 | 488 | 100 | 85 | 92 |
| | | | | | Starch | | | | | | | |
| 120802 | Superfilm No. 4 | 1.5(2%) | 10.0 | 56 | 20.0 | 42.7 | 214 | 33.8 | 405 | 90 | 93 | 101 |
| 120803 | | | 6.0 | 60 | 19.0 | 42.4 | 223 | 39.4 | 425 | 110 | 97 | 98 |
| 120804 | | | 4.0 | 60 | 18.0 | 38.8 | 216 | 35.0 | 375 | 90 | 92 | 102 |
| 120805 | | | 2.0 | 59 | 19.6 | 36.9 | 188 | 17.5 | 336 | 78 | 94 | 99 |

but were used with rather mediocre results. Enzyme conversion of starch has made broad advances in recent years and very greatly improved products are now available. It is believed that many of the ideas and theories applied to starch enzymes may find counterparts in mannogalactan enzyme conversion. A brief summary of the developments in starch enzyme technology may clarify this statement.

Brief Review of Starch Enzyme Technology

It has been found that there are essentially two types of enzymes which attack starch and are commercially important. These have been called α-amylase and β-amylase and usually occur naturally as a mixture. The properties of these enzymes, such as relative amounts of α and β components, thermostability, optimum pH, and potency, differ considerably with the source. The type of action on the starch polymers, however, is much the same for each respective kind of enzyme in the mixture irregardless of source. Techniques have been devised for separating α and β amylases in pure form. It has been demonstrated that the α-amylase attacks the starch chains in a more or less random manner with very little apparent sugar formation during the early stages of enzyme action. The β-amylase on the other hand has been found to begin the attack only at the non-reducing end of the polymer chain and to progressively hydrolyze off two glucose residues at a time in the form of maltose, thereby, saccharifying a considerable part of the starch while the viscosity is being lowered to the desired point. It has, therefore, been found desirable for starch conversion purposes to produce an enzyme product consisting predominantly of α-amylase rather than β-amylase. Several methods of doing this have been utilized by enzyme manufacturers.

1. Bacterial enzymes grown under proper conditions were found to be predominantly α-amylase and in addition had a high degree of thermostability--a very desirable property in starch conversion.

2. Mold and malt enzyme mixtures were treated at a temperature of 70° C. and pH of 6 to 7 for 15 minutes which destroyed the β-amylase.

3. It was found that the presence of calcium ion at a concentration of 0.05*N* greatly stabilizes the α-amylase enzyme and makes the β-amylase considerably more unstable.

Discussion of Mannogalactan Conversions

As mentioned before, it is believed that several factors found to be important in amylase investigation will give valuable leads in enzymic studies on guar. With this in mind it seemed quite desirable to cultivate the bacterial enzyme of Bacillus X. The organism in this case seems to be very well adapted toward growing on locust bean gum and with slight modifications will no doubt produce an enzyme of optimum quality from a conversion standpoint for locust bean gum; however, our prime interest is in the conversion of guar mucilage, and it was a great disappointment to find that the enzyme found to be so potent on locust bean gum did not readily attack guar. It was disappointing again to find that Bacillus X does not grow rapidly on guar and only partially liquifies this mucilage. The organism is able to exist on guar but it does not possess the ability to hydrolyze the polysaccharide. From appearances of viscosity change, part of the polymeric chain is attacked and then the action stops. Attempts made, thus far, to adapt the organism to guar by growing on guar-locust bean gum mixtures have been unsuccessful. It is probable that the linkage of the mannan or galactan chains in guar differs sufficiently from that in locust bean gum to prevent the action taking place beyond a certain point in the chain. This same enzyme (from locust bean gum) was tried in a qualitative way on other species of mannogalactan and found to hydrolyze flame tree and honey locust mucilage rapidly but tara mucilage not at all.

Other bacterial organisms which might grow on guar and produce a potent enzyme were sought but, thus far, none have been found. This phase of the work has, however, not been very extensive.

The Mold Enzyme

Enzymes from molds are usually much more versatile as to type of substrate attacked because a larger variety of enzymes are developed. Thus, the Brown mold grows readily upon guar and produces an enzyme mixture which attacks guar, tara, and locust bean gum almost equally as well. However, the enzymes from molds have certain disadvantages which may or may not become important as the work progresses. (1) They are quite heat labile. (2) They take longer to produce--the mold organism grows more slowly than bacteria. (3) They have a much greater tendency to produce sugar. (4) They are probably more difficult to purify in a simple manner because of the large number of enzymes present.

The fact that the mold enzymes are not always isolated at the same pH may be an important factor insofar as sugar production is concerned. For example, enzyme No. 97 had a pH of 3.6 when in dilute form and after concentration a pH of 3.4. This low pH value may possibly have destroyed a significant part of the liquifying enzyme similar to the α enzyme in amylase mixtures. On this basis, enzymes No. 97-2 and No. 97-3 should contain much greater amounts of this enzyme. Experiments should be made to determine whether or not these factors are important.

It should be mentioned that vacuum concentration of the dilute enzymes has been found to be the best method of preserving their potency over considerable periods of time. Concentrates of several enzymes have been stored for several months in a refrigerator without noticeable loss of potency.

Future Work

1. Bacterial Enzymes

   a. Attempts are being made to adapt Bacillus X to growing and producing an enzyme when placed on guar.

   b. Other bacteria will be sought which will grow and produce enzymes on guar.

   c. A further study of enzyme No. 26 will be made in respect to sugar production.

2. Mold Enzymes

   a. A study of the rate of decrease in viscosity versus sugar production is being made at different pH values to determine whether conditions can be found which will enable a greater reduction in viscosity per unit of sugar produced than the conditions employed in the tubsize experiments of this report.

   b. Various conditioning treatments should be given to the enzyme preparations in an attempt to inactivate a possible saccharifying enzyme and allow the liquifying enzyme to remain active.

   c. The mold should be grown in the presence of media buffered at a somewhat higher pH, for example, 7.0-8.0.

   d. Calcium ion should be added to the substrate in addition to calcium carbonate buffer.

   e. Enzyme No. 89 should be evaluated further.

3. A standardized method of measuring activity should be devised which will give a more consistent measure of enzyme potency in terms of some standard, easily recognized unit.
4. The possible nutrient materials for growing enzymes on a commercial scale should be sought and used in future laboratory preparations. These substances may considerably change the type of enzyme produced.

vg

# PROJECT REPORT FORM

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Notebook 631
Pages 7 - 33

PROJECT NO. 849
COOPERATOR Institute
REPORT NO. 15
DATE Aug. 30, 1945. Typed 9/8/45
NOTE BOOK 603
PAGE 129 TO 158
SIGNED John W. Swanson
John W. Swanson

HANDSHEET STUDIES ON FORMATION QUALITIES OF SEVERAL MANNOGALACTAN MUCILAGES

## INTRODUCTION

The formation of a sheet of paper is the property determined by the degree of uniformity of fiber distribution which is evident when the sheet is viewed by transmitted light. Good formation is important not only for good printing quality but also because of its influence on strength and many other sheet properties.

During the papermaking operation, there are many factors which influence formation to a marked extent. Among the more important variables may be mentioned the kind, consistency, temperature and freeness of the furnish; the type of beating, length of fiber, machine speed, wire mesh, the dandy roll and machine shake.

One of the many desirable characteristics of mannogalactan mucilages is their marked ability to improve formation when added to the pulp. This is accomplished by delaying the natural tendency of the fibers to flocculate or clot so that the sheet is formed from a better dispersed system of pulp. Although there are some indications that this phenomenon may be a combination of hydration and electrostatic charge, very little is known about the fundamental mechanism or the variables which influence this property of mucilage.

Evidence accumulated from several mill trials of guar mucilages G4-2 and G4-6 has shown the formation benefit to be rather irregular--sometimes giving fairly good improvement and other times apparently none at all. It became desirable therefore, to make a study of several mannogalactan products to determine their relative effectiveness and some of the variables involved in the formation quality.

The first problem in the investigation was to devise a suitable method whereby valid differences between mucilages could be demonstrated. It was believed that the many variables connected with the usual handsheet-making procedure might invalidate conclusions drawn from such studies. Therefore, the problem was attacked from two angles--(a) By devising a handsheet technique giving reproducible valid results, and (b) A dynamic method of measuring flocculation of pulps independent of many handsheet variables. Mr. Truttschel has undertaken the dynamic study and the writer has attempted to improve the handsheet method of evaluation of formation.

EXPERIMENTAL

Work Done:

1. A handsheet-method of evaluating the relative formation quality of mucilages was devised which is believed to give valid results.

2. Several mucilages were compared with the above handsheet technique.

3. Several experiments were made to determine the effect of colloidal iron on formation quality.

4. A preliminary investigation of the effects of beating, consistency, time of flocculation and presence of rosin and alum on formation was made.

Method of Making Formation Handsheets

Small differences in the formation qualities of mucilages could not be determined by the regular methods of making handsheets. It was decided therefore, to exaggerate the flocculation effects in two ways--(a) A very lightly beaten pulp was used, and (b) the pulp was allowed to flocculate in the deckel box for a longer time before forming the sheet. Experiments with increasing flocculation intervals gave poorer formation with time both in the presence and absence of mucilage. In the absence of mucilage up to 35 seconds, the formation became poorer quite rapidly and then slower from 40-90 seconds. It was decided that a flocculation time of 35 seconds in the deckel box would show sufficient differences in formation qualities of mucilages to overcome many of the uncontrollable errors involved in sheetmaking.

Beating Procedure

Three hundred-sixty grams (O.D. basis) of Weyerhaeuser wartime bleached sulfite pulp were placed in the Valley beater with 23 liters of water. After soaking for 5-10 minutes, the pieces of pulp were pulled apart by hand and slushed with the beater roll without weight on the bedplate for 5 minutes. The balance weight and 4500-gram weight were then added and the beating continued for exactly 10 minutes. The stock was stored in a covered 10-gallon crock and any remaining after 48 hours was discarded.

Handsheet Making Procedure

Forty grams of pulp (O.D. basis) were measured out in a graduated cylinder and placed in a 4-gallon enamelled bucket. The pulp was vigorously agitated by means of a Lightnin' mixer and the desired quantity of cooked mucilage slowly added. Two minutes after the mucilage addition, the stirring was stopped and the mixture allowed to stand for three minutes. Dilution water was added to make a stock consistency of 0.5%, the mixture was stirred for five minutes and the handsheets were made in the following manner:

The deckel box was marked with a red line at a volume of 3.5 liters in order to maintain the consistency as constant as possible. Three hundred milliliters of diluted stock were measured out in a graduated cylinder while the deckel box was being filled to the mark with water. The stock was added and immediately stirred four vigorous strokes and one slow stroke with the deckel box stirrer. When the stirrer was removed, a Kodak timer was started and after 35 seconds, the valve was released and the sheet formed. Two new blotters were placed upon the sheet and held down by hand for several seconds. The sheet was couched from the wire and one new blotter placed on the wire side. The top blotter was removed, the sheet was turned wire side up and placed in the press. An additional blotter was placed on the top and the bottom of the pile, and the sheets were pressed at 100 pounds per square inch for one minute. The sheets were labelled on the wire side and dried on the felt side blotter on a hotplate at 230° F. for seven minutes. Eight sheets were made for each set. Blank sheets were made each day and for each beater run. The basis weight of sheets was held as constant as possible although small variations in this were found to not seriously affect the formation value. In several cases 4.5% of alum was added to the remaining 0.5% stock and an additional set of four sheets was made in a similar manner. Sufficient alum was also added to the deckel box prior to stirring to maintain the pH at about 5.0.

The experiments on effects of colloidal iron were carried out with sols made by hydrolysis of ferric chloride and ferrous ammonium sulfate in boiling water. These were diluted to one per cent iron and added to the pulp just before the mucilage and dilution water.

Procedure for Making Handsheets at Various Consistencies

A sheet metal extension was made for the handsheet deckel box of such capacity that it was possible to form a 1.5-gram handsheet from 15 liters of 0.01% stock. The procedure of making handsheets was otherwise the same as before with the exception that various time intervals of flocculation were used.

A series of sheets at various consistencies at freeness 600 were also made. This stock was beaten for 51 minutes.

Experiments with rosin and various amounts of alum were made at freeness 850. The pH in the sheet mold was maintained at about 5 by addition of 3N sulfuric acid.

COOKING OF MUCILAGES

Borax Cook Method

Two and one-half grams of mucilage were added to 300 ml. of 0.25% borax solution in a 600-ml. beaker. Steam was injected until the temperature was 85° C., whereupon, sufficient hydrochloric acid was added to lower the pH to 4.5-5.5. After holding at 80° C. for 15 minutes, the mixture was diluted to 0.5% mucilage and placed in a 500-ml. Erlenmeyer flask.

Tap Water Cook

The above cooking procedure was followed except that borax and acid were not used. Further deviations in cooking procedure were necessary in some cases and these will be explained as they occur.

REPRODUCIBILITY OF RESULTS

It was necessary to follow the sheetmaking procedure quite exactly in order to obtain consistent results. Even then an occasional set of sheets did not check very well. It was also necessary to allow the water used for beating the pulp and making the handsheets to run to constant temperature each time and to remove excess iron from the pipes. This is now believed to have caused many of the erratic results in earlier experiments.

The formation values were determined on five of the best sheets chosen from the original 8 of each set. Any sheets having black spots or other faults were discarded wherever possible.

TABLE I

REPRODUCIBILITY OF FORMATION FROM PULPS OF TEN BEATER RUNS

| Beater Run No. | per cent Locust Bean Gum | FORMATION VALUE Max. | Min. | Av. |
|---|---|---|---|---|
| 1 | Blank | 21.4 | 19.1 | 20.3 |
| 2 | Blank | 24.8 | 20.0 | 22.8 |
| 3 | Blank | 23.1 | 21.2 | 22.2 |
| 4 | Blank | 23.4 | 21.7 | 22.6 |
| 5 | Blank | 24.0 | 21.4 | 22.8 |
| 6 | Blank | 23.6 | 20.0 | 22.4 |
| 7 | Blank | 23.6 | 21.4 | 22.3 |
| 8 | Blank | 22.1 | 19.4 | 21.3 |
| 9 | Blank | 26.6 | 20.5 | 23.8 |
| 10 | Blank | 22.3 | 19.8 | 21.0 |
| 1 | 0.1 | 27.2 | 23.8 | 25.6 |
| 6 | 0.1 | 26.9 | 24.0 | 26.0 |
| 1 | 1.5 | 37.9 | 34.2 | 35.9 |
| 6 | 1.5 | 36.8 | 35.2 | 36.0 |

PRESENTATION OF DATA

The formation data of handsheets made with various kinds and quantities of mucilages are presented in Tables II to IX and Figures 1 to 4.

The data on the effects of consistency, beating, and rosin and alum on formation are presented in Tables X to XII and Figures 5 to 7. These are discussed in a separate section.

TABLE II

FORMATION VALUES OF HANDSHEETS CONTAINING LOCUST BEAN GUM AT VARIOUS CONCENTRATIONS

| IPC File No. | Per Cent Mucilage Added | FORMATION VALUE Max. | Min. | Av. |
|---|---|---|---|---|
| 116101 | Blank | 21.4 | 19.1 | 20.3 |
| 116102 | 0.078 | 27.2 | 23.8 | 25.6 |
| 116104 | 0.15 | 29.1 | 26.0 | 27.7 |
| 116105 | 1.0 | 35.2 | 34.2 | 34.6 |
| 116106 | 1.5 | 37.9 | 34.2 | 35.9 |
| 116107 | 2.0 | 39.7 | 35.7 | 37.3 |
| | Check Run on Locust Bean Gum | | | |
| 116591 | Blank | 23.6 | 21.4 | 22.3 |
| 116593 | 0.1 | 26.9 | 24.0 | 26.0 |
| 116595 | 0.75 | 34.2 | 32.9 | 33.6 |
| 116597 | 1.5 | 36.8 | 35.2 | 36.0 |
| 116592 | Blank* | 21.6 | 19.1 | 20.2 |
| 116594 | 0.1* | 22.1 | 19.8 | 20.9 |
| 116596 | 0.75* | 22.1 | 19.5 | 20.7 |
| 116598 | 1.5* | 22.9 | 19.7 | 21.5 |
| | Borax-Cooked Locust Bean Gum | | | |
| 116599 | 0.1 | 20.5 | 16.0 | 19.1 |
| 116601 | 0.75 | 33.3 | 30.5 | 31.8 |
| 116603 | 1.5 | 37.9 | 32.5 | 36.1 |
| 116605 | -- | 22.1 | 19.4 | 21.3 |
| 116872 | Blank | 22.7 | 19.7 | 21.0 |
| 116873 | .05% | 24.5 | 22.7 | 23.4 |
| 116874 | 0.1 | 25.8 | 24.3 | 25.1 |
| 116875 | 0.2 | 28.1 | 25.3 | 26.9 |
| 116876 | 0.4 | 31.3 | 28.1 | 29.9 |
| 116877 | 0.75 | 33.3 | 31.6 | 32.6 |

* 4.5% alum added to remaining stock.

TABLE III

FORMATION VALUES OF HANDSHEETS CONTAINING GUAR MUCILAGES
G4-6, G4-2, G4 over 8xx and G44

| IPC File No. | Kind of Mucilage Added | Per Cent Mucilage Added | FORMATION VALUE Max. | Min. | Av. |
|---|---|---|---|---|---|
| 116129 | Blank | -- | 24.8 | 20.0 | 22.8 |
| 116130 | G46 | 0.1 | 26.3 | 24.8 | 25.4 |
| 116131 | G46 | 0.75 | 29.1 | 27.5 | 28.3 |
| 116132 | G46 | 1.5 | 31.3 | 27.8 | 29.8 |
| 116233 | Blank | -- | 23.4 | 21.7 | 22.6 |
| 116234 | G42 | 0.1 | 26.9 | 24.8 | 25.3 |
| 116235 | G42 | 0.75 | 27.8 | 26.0 | 27.1 |
| 116237 | G42 | 1.5 | 31.3 | 28.4 | 29.4 |
| 116154 | G4 | 0.1 | 26.3 | 24.5 | 25.4 |
| 116155 | G4 | 0.75 | 30.1 | 26.9 | 28.3 |
| 116156 | G4 | 1.5 | 30.5 | 27.8 | 28.5 |
| | | Check on G4 | | | |
| 117349 | Blank | -- | 23.6 | 20.5 | 22.1 |
| 117350 | G4 | 0.1 | 26.3 | 22.3 | 24.2 |
| 117351 | G4 | 0.75 | 27.8 | 26.0 | 27.1 |
| 117352 | G4 | 1.5 | 30.9 | 28.4 | 29.8 |
| 117355 | Blank | -- | 23.8 | 22.3 | 23.0 |
| 117356 | G44 | 0.1 | 25.3 | 24.0 | 24.9 |
| 117357 | G44 | 0.75 | 30.1 | 26.6 | 28.2 |
| 117358 | G44 | 1.5 | 29.4 | 27.8 | 28.6 |

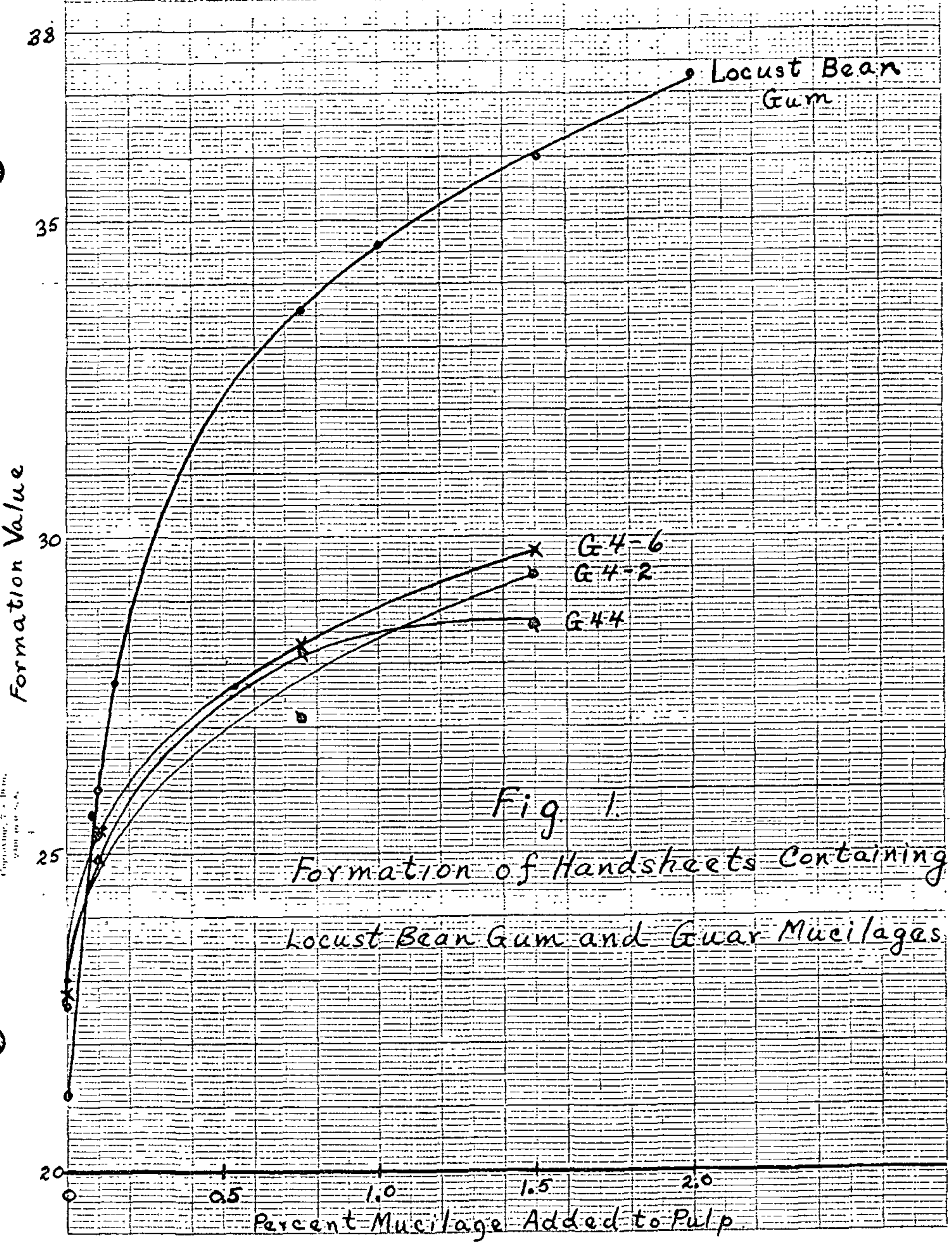



Fig. 1.
Formation of Handsheets Containing Locust Bean Gum and Guar Mucilages

TABLE IV

FORMATION VALUES OF HANDSHEETS CONTAINING GUAR MUCILAGES
G4-H and G4-L
(Borax Cooked)

| IPC File No. | Kind of Mucilage Added | Per Cent Mucilage Added | FORMATION VALUE | | |
|---|---|---|---|---|---|
| | | | Max. | Min. | Av. |
| 116259 | Blank | — | 23.6 | 20.0 | 22.4 |
| 116267 | G4-H | 0.1 | 26.0 | 22.1 | 24.4 |
| 116268 | G4-H | 0.75 | 29.8 | 26.6 | 28.0 |
| 116269 | G4-H | 1.5 | 28.1 | 26.3 | 27.2 |
| 116273 | G4-L | 0.1 | 24.5 | 21.9 | 22.9 |
| 116274 | G4-L | 0.75 | 28.4 | 24.3 | 25.5 |
| 116275 | G4-L | 1.5 | 26.0 | 22.3 | 24.6 |
| G4-H and G4-L Cooked in Plain Water | | | | | |
| 116627 | Blank | — | 26.6 | 20.5 | 23.8 |
| 116621 | G4-H | 0.1 | 27.5 | 24.0 | 26.2 |
| 116623 | G4-H | 0.75 | 31.3 | 29.1 | 30.0 |
| 116625 | G4-H | 1.5 | 32.5 | 30.5 | 31.3 |
| 116629 | G4-L | 0.1 | 25.8 | 23.4 | 24.8 |
| 116631 | G4-L | 0.75 | 30.5 | 26.6 | 28.0 |
| 116633 | G4-L | 1.5 | 29.1 | 28.1 | 28.5 |

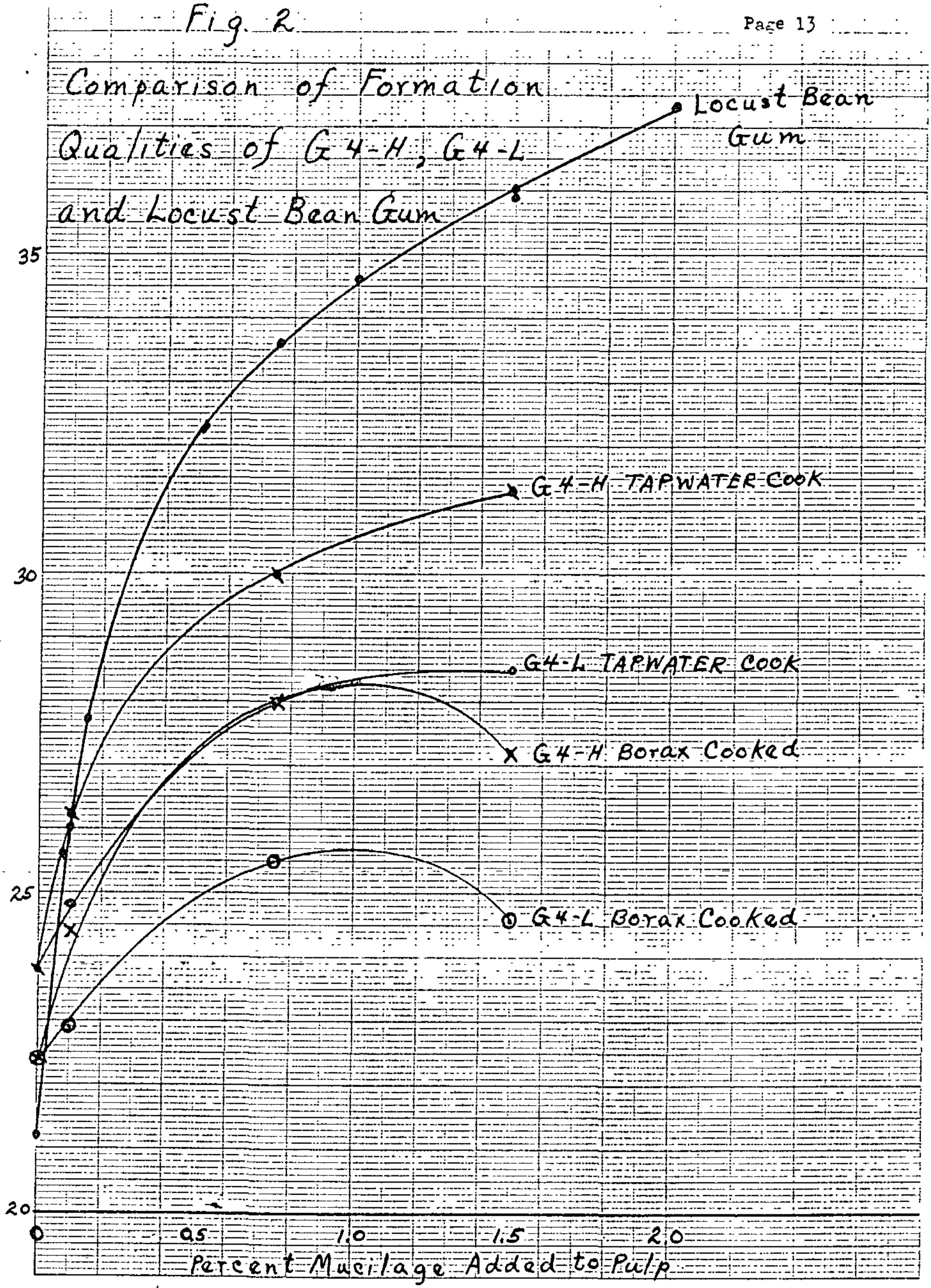


Fig. 2
Comparison of Formation Qualities of G4-H, G4-L and Locust Bean Gum
Locust Bean Gum
G4-H TAPWATER COOK
G4-L TAPWATER COOK
G4-H Borax Cooked
G4-L Borax Cooked
Formation Value
35
30
25
20
0
0.5
1.0
1.5
2.0
Percent Mucilage Added to Pulp

TABLE V

FORMATION VALUES OF HANDSHEETS CONTAINING GUAR MUCILAGES
SPECIAL G4-6, "High Mannose Guar" and Acid-cooked G4-6

| IPC File No. | Kind of Mucilage Added | Per Cent Mucilage Added | FORMATION VALUE Max. | Min. | Av. |
|---|---|---|---|---|---|
| 116605 | Blank | -- | 22.1 | 19.4 | 21.3 |
| 116607 | Special G4-6 | 0.1 | 24.0 | 21.2 | 22.9 |
| 116609 | Special G4-6 | 0.75 | 28.1 | 26.0 | 27.0 |
| 116611 | Special G4-6 | 1.5 | 30.9 | 28.1 | 29.7 |
| 116613 | High Mannose Guar | 0.1 | 27.2 | 25.0 | 26.3 |
| 116615 | High Mannose Guar | 0.75 | 30.1 | 27.8 | 29.0 |
| 116617 | High Mannose Guar | 1.5 | 32.5 | 30.1 | 31.0 |
| 117373 | Blank | -- | 22.5 | 18.9 | 21.5 |
| 117380 | G4-6 Acid Cooked | 0.1 | 25.0 | 24.8 | 24.9 |
| 117381 | G4-6 Acid cooked | 0.75 | 27.5 | 25.0 | 25.8 |
| 117382 | G4-6 Acid cooked | 1.5 | 30.5 | 26.9 | 28.2 |

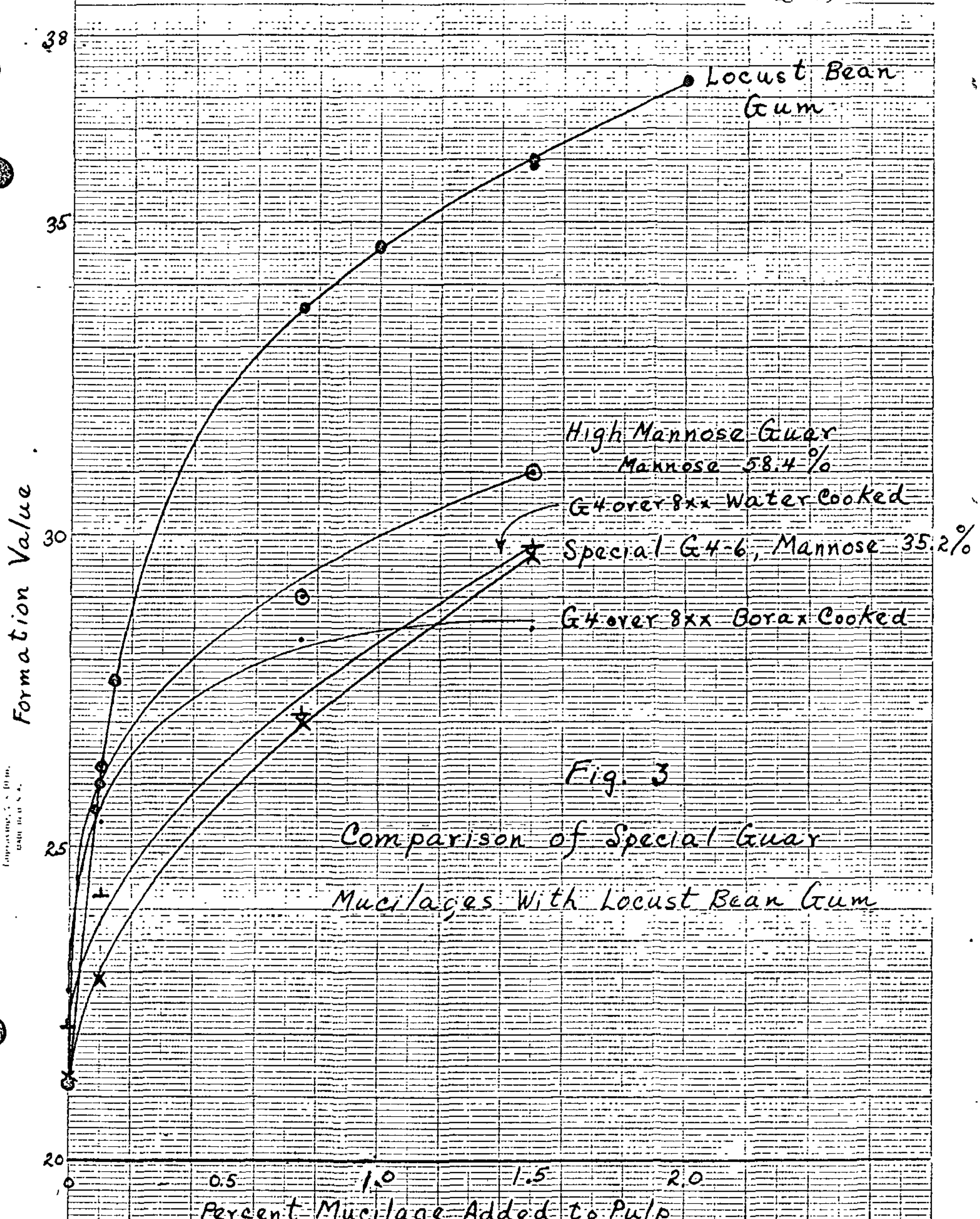



Fig. 3

Comparison of Special Guar Mucilages With Locust Bean Gum

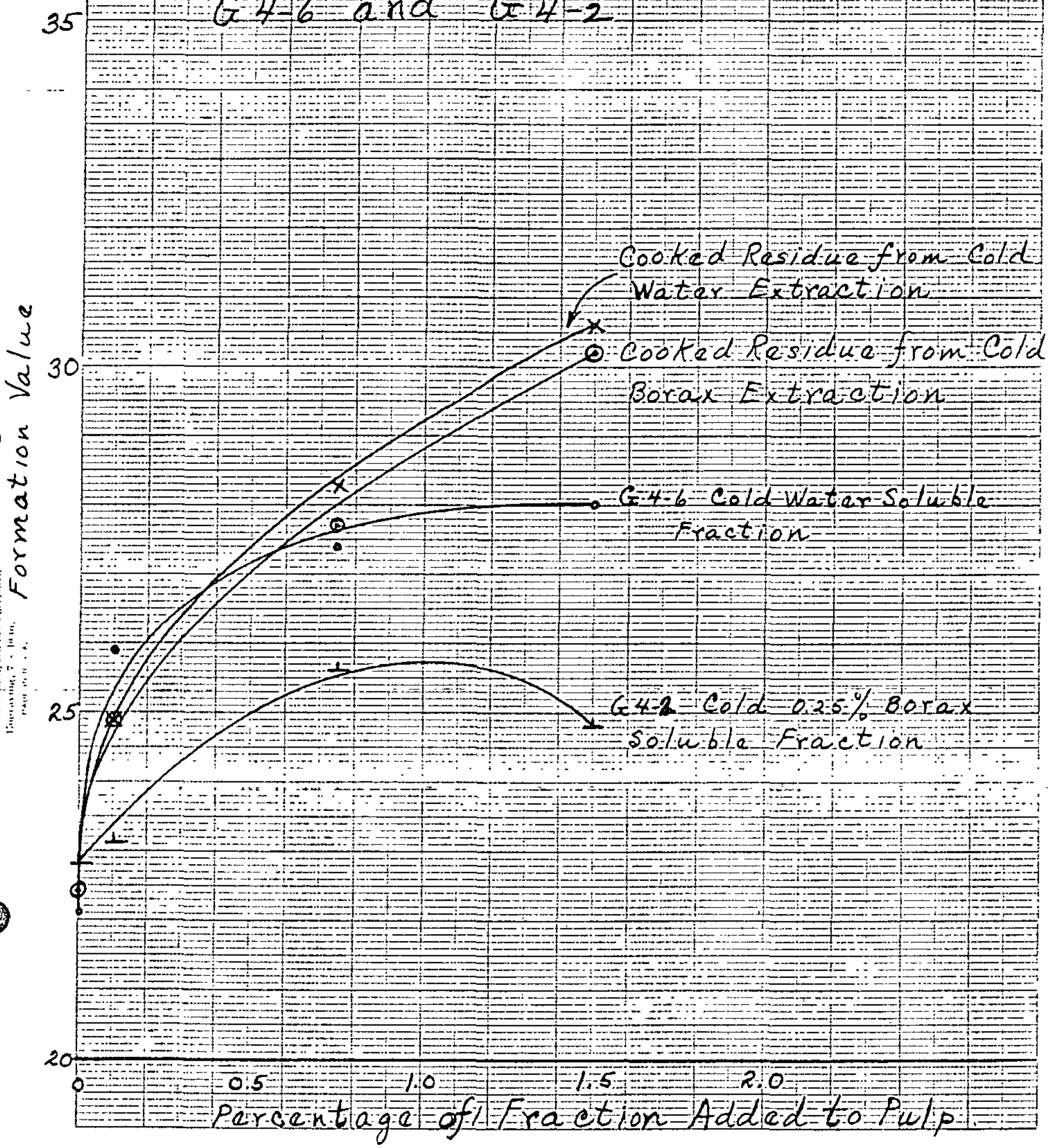
TABLE VI

FORMATION VALUES OF HANDSHEETS CONTAINING VARIOUS FRACTIONS OF GUAR MUCILAGES G4-6 and G4-2

| IPC File No. | Kind of Mucilage Used | Type of Extraction | Fraction Used | % Added To Pulp | FORMATION VALUE Max. | Min. | Av. |
|---|---|---|---|---|---|---|---|
| 116145 | Blank | — | — | — | 23.1 | 21.2 | 22.2 |
| 116146 | G4-6 | Water | Cold water Soluble | 0.1 | 28.7 | 24.3 | 25.9 |
| 116147 | G4-6 | Water | Cold water Soluble | 0.75 | 27.8 | 27.2 | 27.4 |
| 116148 | G4-6 | Water | Cold water Soluble | 1.5 | 29.4 | 25.8 | 28.0 |
| 116149 | G4-6 | Water | Cooked Residue | 0.1 | 27.8 | 23.8 | 24.9 |
| 116150 | G4-6 | Water | Cooked Residue | 0.75 | 29.4 | 26.9 | 28.3 |
| 116151 | G4-6 | Water | Cooked Residue | 1.5 | 32.9 | 29.1 | 30.6 |
| 116238 | Blank | — | — | — | 24.0 | 21.4 | 22.8 |
| 116239 | G4-2 | 0.25% Borax | Cold Borax Soluble | 0.1 | 23.8 | 22.5 | 23.1 |
| 116240 | G4-2 | 0.25% Borax | Cold Borax Soluble | 0.75 | 26.9 | 25.0 | 25.6 |
| 116241 | G4-2 | 0.25% Borax | Cold Borax Soluble | 1.5 | 25.8 | 23.1 | 24.8 |
| 116259 | Blank | — | — | — | 23.6 | 20.0 | 22.4 |
| 116260 | G4-2 | 0.25% Borax | Cooked Residue | 0.1 | 25.8 | 23.4 | 24.9 |
| 116261 | G4-2 | 0.25% Borax | Cooked Residue | 0.75 | 28.7 | 26.6 | 27.7 |
| 116262 | G4-2 | 0.25% Borax | Cooked Residue | 1.5 | 30.9 | 29.8 | 30.2 |

Fig. 4

A Comparison of the Formation Qualities of Various Fractions of G4-6 and G4-2



## TABLE VII

### EFFECT OF 4.5% OF ALUM ON THE FORMATION QUALITY OF SEVERAL MUCILAGES

| IPC File No. | Kind of Mucilage Added | Per Cent Mucilage Added | FORMATION VALUE Max. | Min. | Av. |
|---|---|---|---|---|---|
| 116591 | Blank | No Alum | 23.6 | 21.4 | 22.3 |
| 116592 | Blank + Alum | | 21.6 | 19.1 | 20.2 |
| 116594 | LBG-W* | 0.1 | 22.1 | 19.8 | 20.9 |
| 116596 | LBG-W | 0.75 | 22.1 | 19.5 | 20.7 |
| 116598 | LBG-W | 1.5 | 22.9 | 19.7 | 21.5 |
| | Borax-Cooked Locust Bean Gum | | | | |
| 116600 | LBG-B | 0.1 | 19.2 | 18.1 | 18.5 |
| 116602 | LBG-B | 0.75 | 21.6 | 20.5 | 21.0 |
| 116604 | LBG-B | 1.5 | 21.2 | 19.1 | 20.3 |
| 116263 | Blank + Alum | | 20.3 | 18.0 | 19.3 |
| 116270 | G4-H | 0.1 | 17.6 | 15.7 | 16.6 |
| 116271 | G4-H | 0.75 | 17.1 | 15.0 | 16.4 |
| 116272 | G4-H | 1.5 | 16.3 | 14.9 | 15.7 |
| 116276 | G4-L | 0.1 | 18.5 | 17.1 | 17.7 |
| 116277 | G4-L | 0.75 | 16.7 | 16.0 | 16.3 |
| 116278 | G4-L | 1.5 | 16.3 | 14.9 | 15.7 |
| 116606 | Blank + Alum | | 22.5 | 19.1 | 20.5 |
| 116608 | Sp. G4-6 | 0.1 | 20.8 | 19.1 | 20.2 |
| 116610 | Sp. G4-6 | 0.75 | 21.9 | 19.1 | 20.0 |
| 116612 | Sp. G4-6 | 1.5 | 22.9 | 19.1 | 20.3 |
| 116614 | High Mannose-Guar | 0.1 | 22.3 | 20.5 | 21.4 |
| 116616 | High Mannose-Guar | 0.75 | 21.6 | 19.8 | 21.0 |
| 116618 | High Mannose-Guar | 1.5 | 20.5 | 18.7 | 19.6 |
| 116242 | Blank + Alum | | 23.4 | 19.1 | 20.9 |
| 115243 | Borax Soluble G4-2 | 0.1 | 22.1 | 18.9 | 20.7 |
| 116244 | Borax Soluble G4-2 | 0.75 | 22.3 | 19.4 | 20.6 |
| 116245 | Borax Soluble G4-2 | 1.5 | 22.1 | 20.8 | 21.4 |
| 116263 | Blank + Alum | | 20.3 | 18.0 | 19.3 |
| 116264 | Borax Residue Cooked | 0.1 | 19.7 | 18.2 | 19.2 |
| 116265 | Borax Residue Cooked | 0.75 | 18.4 | 16.8 | 17.4 |
| 116266 | Borax Residue Cooked | 1.5 | 18.9 | 16.6 | 17.8 |

* Cooked in tep water.

TABLE VIII

EFFECTS OF COLLOIDAL IRON AND IRON-CONTAINING MATERIALS ON THE FORMATION VALUE

| IPC File No. | Description of Experiment | THWING FORMATION VALUE Max. | Min. | Av. |
|---|---|---|---|---|
| 117359 | Blank | 22.3 | 20.5 | 21.8 |
| 117360 | 0.75% LBG | 37.3 | 33.8 | 34.8 |
| 117361 | 20 p.p.m. colloidal ferric oxide in water + 0.75% LBG | 26.3 | 24.8 | 25.4 |
| 117362 | 2 p.p.m. colloidal ferric oxide in water + 0.75% LBG | 35.2 | 32.1 | 33.5 |
| 117369 | Blank | 22.3 | 21.0 | 21.7 |
| 117370 | 1.5% LBG cooked in $H_2O$ containing 10 p.p.m. colloidal ferric oxide | 36.8 | 35.2 | 36.4 |
| 117371 | 1.5% LBG cooked in $H_2O$ containing 30 p.p.m. colloidal ferric oxide | 37.3 | 35.7 | 36.4 |
| 117545 | 1.5% LBG | 35.7 | 34.7 | 35.3 |
| 117549 | 10 p.p.m. colloidal ferrous iron in $H_2O$ + 1.5% LBG | 18.4 | 15.7 | 16.8 |
| 117550 | 2 p.p.m. colloidal ferrous iron in $H_2O$ + 1.5% LBG | 28.7 | 22.1 | 26.1 |
| 117551 | 0.5 p.p.m. colloidal ferrous iron in $H_2O$ + 1.5% LBG | 33.8 | 32.1 | 32.8 |
| 117552 | Blank | 22.3 | 19.8 | 21.0 |
| 117558 | 1.5% LBG | 36.8 | 34.2 | 35.6 |
| 117559 | 1.5% LBG cooked in presence of 20 p.p.m. colloidal ferrous iron | 37.9 | 33.3 | 35.5 |
| 117363 | 0.75% LBG + 25% guar seed coat | 35.7 | 32.9 | 34.9 |
| 117367 | 0.75% LBG + 25% G4-2 dust | 35.7 | 32.5 | 33.7 |
| 117368 | 1.5% LBG + 25% G4-2 dust | 37.9 | 35.7 | 36.4 |
| 117373 | Blank | 22.5 | 18.9 | 21.5 |
| 117375 | 0.75% LBG | 35.7 | 32.1 | 34.3 |
| 117377 | 0.75% LBG + 10% guar pigment | 36.2 | 31.3 | 33.2 |
| 117378 | 0.75% LBG + 10% cooked pigment | 35.7 | 32.1 | 33.3 |
| 117379 | 0.75% LBG + 42.5% cooked pigment | 35.7 | 32.9 | 34.4 |

## DISCUSSION OF DATA COMPARING VARIOUS MUCILAGES

The data in Tables II to V and Figures 1 to 3 indicate that guar mucilages do not possess as good a formation-improving quality as does locust bean gum. This deficiency is not entirely due to a lower purity of mannogalactan since a correction for 30-40% of inert material still shows the active mucilage to be inferior. The quite highly purified guar mucilages G-44, G4-H, and G4-L were also significantly poorer than locust bean gum.

The curves for borax-cooked G4-H and G4-L show a poorer formation at 1.5% mucilage addition than at 0.75%. This behavior cannot be explained. In fact, visual examination of these two sets of sheets shows the 1.5% addition to be slightly better than 0.75%. This is the only case which has been noted where the Thwing formation tester did not agree with visual examination of the sheets.

A comparison of a "High Mannose" guar mucilage (mannose 58.4%) with G4-6 mucilage (Mannose 35.2%) indicated that the mannose content affects the formation quality to some extent. However, the "High Mannose" mucilage is definitely poorer than locust bean gum under the conditions used in these experiments. Some difficulty was experienced in dispersing the "High Mannose" guar because of the large particle size. It was necessary to cook the mucilage at 80-85° C. for 45 minutes, treat in a Waring Blendor for 5 minutes, and follow with another 10 minutes heating at 80° C. This gave a pretty fair dispersion.

The curves in Figure 2 comparing G4-H, G4-L, and locust bean gum indicate that borax cooking of the mucilage may be detrimental to the formation quality. Attempts to check the borax effect on locust bean gum gave several rather erratic results but examination of all the data did not indicate that borax cooking was detrimental to the formation quality of this mucilage. The exact pH of the borax cooks are not known but they were all in the range of 4-5.5. Further work on the effect of the cooking pH on formation has been done by Mr. Truttschel with the Flocculation Tester. Several of the mucilages which he used in this study were incorporated into handsheets (see Table IX). The pH of the borax cook did not appreciably influence the formation. One mucilage, cooked in water containing enough sodium hydroxide to provide a pH of 11.2, did lower the formation significantly. However, the mucilage was incompletely dispersed at this pH.

Several experiments were made to determine whether or not a part of the guar mucilage was detrimental or exceedingly poor in formation quality. G4-6 mucilage was extracted with cold water at 0.5% concentration and the solids in the extract determined. The extract was evaluated for formation-improving quality in the regular manner. The undissolved residue of G4-6 was cooked in the usual way and also evaluated. These data plotted in Figure 4 indicate that the cold water-soluble fraction is inferior to the undissolved residue at higher concentrations but equally as good at lower concentrations.

Another type of fractionation was made on G4-2 by stirring 2.5 g. of the mucilage in 300 ml. of 0.25% borax for one hour. Previous work had shown that roughly 20% of the original weight of mucilage dissolved and this figure was used to calculate the solids. The residue was acidified and cooked in the usual way. The borax-soluble fraction was added to the pulp without cooking and was found to be distinctly inferior in formation quality to the cooked residue which compared favorably with whole cooked G-46 mucilage. It is believed that the principal constituent of the borax-soluble fraction was protein.

The addition of 4.5% of alum to the stock (no rosin) caused the sheets to have a much poorer formation (see Table VII). In a number of cases, a trend can be noted wherein the formation became poorer with increasing quantity of mucilage and attained a value considerably below the blank. These data seem to support the hypothesis that formation improvements of mucilages are at least partially due to a negative electrostatic charge on the mucilage micelle which becomes neutralized by means of the positively charged alumina.

Colloidal iron present in pulp and water was found to be quite detrimental to the formation qualities of mucilages. For example, sheets made from pulp and water containing 20 p.p.m. of colloidal ferric oxide and 0.75% locust bean gum had a formation value 9.4 points lower than sheets made with tap water. Water containing 2 p.p.m. of colloidal ferric iron had practically no detrimental effect. Colloidal ferrous iron was found to be more potent in decreasing formation quality than ferric iron. As little as 0.5 p.p.m. of ferrous iron in the water caused a noticeable decrease in formation value and 10 p.p.m. of ferrous iron gave a formation value much lower than a blank sheet not containing mucilage.

The discovery that colloidal iron is detrimental to the formation value led to the testing of various mucilages qualitatively for iron. It was found that a rough relationship between the purity of the guar mucilages and intensity of iron stain actually existed. (Potassium ferrocyanide in dil $HNO_3$ was used to test for iron.) Further testing established the fact that the iron was entirely on the surface of the seed coat of guar and the more seed coat the mucilage contained, the more intense was the iron stain. Locust bean gum gave a practically negative test. Several experiments were made to determine whether or not the iron present in the guar seed coat was the cause of the inferior formation quality. Locust bean gum was cooked with both colloidal ferric and ferrous iron, with 25% of ground guar seed coat, 25% G4-2 dust and various quantities of the guar purple pigment and the accompanying iron but in no case did it appear that these materials caused a poorer formation quality (see Table VIII).

Several experiments were made to determine whether or not the guar protein was responsible for a lower formation value. G4-6 mucilage was extracted at 80-85° C. for 10 minutes with dilute borax (2.5 g. G4-6 in 300 ml. of 0.25% borax) and the insoluble part filtered off. The filtrate was used to make a regular G4-6 borax cook and was acidified to a pH of 6.6. A similar cook was made and acidified to a pH of 4.5 and another G4-6 extract was used to cook locust bean gum at pH of 3.65. These experiments did not indicate that the proteinaceous materials of guar were responsible for a lower formation quality.

The colloid milling of a guar G4-2 dispersion did not affect its formation quality.

A dispersion of G4 (over 8xx) was made strongly alkaline with excess sodium hydroxide and added to pulp giving a pH of about 12. Formation handsheets were made and the remaining stock acidified to a pH of 3 and a second set of sheets made at this pH. These two sets of sheets were practically identical in formation value.

Neither ball milling locust bean gum nor oven heating at 107° C. for 12 hours appeared to affect the formation value of the mucilage.

TABLE IX

EFFECTS OF VARIOUS TREATMENTS ON FORMATION QUALITIES OF MUCILAGE

| IPC File No. | Description of Experiment | THWING FORMATION VALUE Max. | Min. | Av. |
|---|---|---|---|---|
| 117365 | 0.75 G4-6 | 28.4 | 26.0 | 27.1 |
| 117366 | 0.75 G4-6 cooked in presence 0.5% sodium hydrosulfite | 28.4 | 24.0 | 27.0 |
| 117553 | 1.5% G4-6 cooked plain water at pH = 7.7 | 30.1 | 26.6 | 28.6 |
| 117554 | 1.5% G4-6 cooked water + NaOH pH = 11.2 | 29.4 | 25.0 | 26.7 |
| 117555 | 1.5% G4-6 Borax cooked pH = 4.85 | 30.9 | 26.6 | 29.0 |
| 117562 | 1.5% G4-6 Borax cooked pH = 3.53 | 30.5 | 26.3 | 28.7 |
| 117563 | 1.5% G4-6 Borax cooked pH = 5.46 | 30.1 | 27.5 | 28.5 |
| 117547 | 1.5% G4-6 + G4-6 Borax extract pH = 4.51 | 28.1 | 27.5 | 27.9 |
| 117548 | 1.5% G4-6 + G4-6 Borax extract pH = 6.6 | 27.8 | 25.3 | 27.1 |
| 117556 | 1.5% LBG + G4-6 Borax extract old pulp | 37.9 | 33.8 | 35.7 |
| 117557 | 1.5% LBG + G4-6 Borax extract new pulp | 37.3 | 34.7 | 35.6 |
| 117561 | 1.5% G4-2 - not colloid milled | 27.8 | 25.0 | 26.5 |
| 117560 | 1.5% G4-2 - colloid milled twice | 27.5 | 24.5 | 26.2 |
| 117351 | 0.75% G4 over 8 xx | 27.8 | 26.0 | 27.1 |
| 117353 | 0.75% G4 over 8xx made alkaline pH about 12 | 28.4 | 25.0 | 27.3 |
| 117354 | Same stock as above made acid $H_2SO_4$ pH about 3 | 27.2 | 26.0 | 26.6 |
| 117372 | 1.5% LBG (Ball milled 360 hours) | 35.7 | 34.7 | 35.4 |
| 117546 | 1.5% LBG Heated dry at 107° C. for 12 hours | 35.2 | 32.9 | 34.3 |
| 117364 | 0.75% LBG + 0.1% waste sulfite liquor | 37.3 | 33.8 | 35.4 |

DISCUSSION OF THE EFFECTS OF CONSISTENCY, BEATING, AND ROSIN AND ALUM ON HANDSHEET FORMATION

Data obtained by Mr. Truttschel in working with the Flocculation Tester indicated that G4-6 mucilage was inferior to locust bean gum when added to dilute stocks but, when the mucilages were added to pulp at beater consistency (similar to present experiments 1.5%), G4-6 mucilage appeared to be just as good as locust bean gum. The latter characteristic could not be confirmed by the handsheet method. Also, Mr. Truttschel found that the pH of the borax cooking of G4-6 had a considerable effect on the formation quality, being poorest at a pH of 5.6. This also could not be confirmed by the handsheet method and these facts made it desirable to determine the reason for the discrepancy of the two methods of measuring relative formation quality.

After considering the conditions of the pulps in the two methods, it was thought that the principal difference existed in the consistency. The Flocculator measurements were made at an average consistency of about 0.0135% while the handsheets were made at approximately 0.04%. It was postulated that the interval before serious floccing of fibers begins is much longer at extreme dilutions of pulp than at higher consistencies. It would follow then that the mucilage would have to work harder to keep pulps dispersed at high than at low consistencies. The differences between the formation quality of mucilages would appear to be less at dilute consistencies and greater at increased consistencies, even though the measurements might be more precise at the dilute consistency.

An extension for the sheet mold was made which enabled the forming of handsheets from stock at 0.01% consistency. Several series of sheets were made at various flocculation times, consistencies and with and without 2% locust bean gum. (This was the amount of mucilage used by Mr. Truttschel.) Also two G4-6 cooks of different pH values were tried at 0.01% consistency to determine whether or not an effect comparable to that of the Flocculator could be noted.

The data of Table X and Figure 5 appear to support the proposed ideas. It may be seen that the difference in formation quality between G4-6 and locust bean gum is much smaller at 0.01% consistency than at 0.03% consistency; especially during the first 30 seconds of flocculation time. Also it may be noted that borax-cooked G4-6 at pH of 5.35 is definitely below G4-6 cooked at pH of 6.9. These data appear to confirm the data obtained with the Flocculation Tester. It appears from these experiments that a somewhat higher consistency than 0.0135 should be used in the Flocculation Tester for evaluating mucilages.

TABLE X

EFFECT OF TIME OF FLOCCULATION AND CONSISTENCY
ON THE FORMATION VALUE OF HANDSHEETS
(Freeness 850)

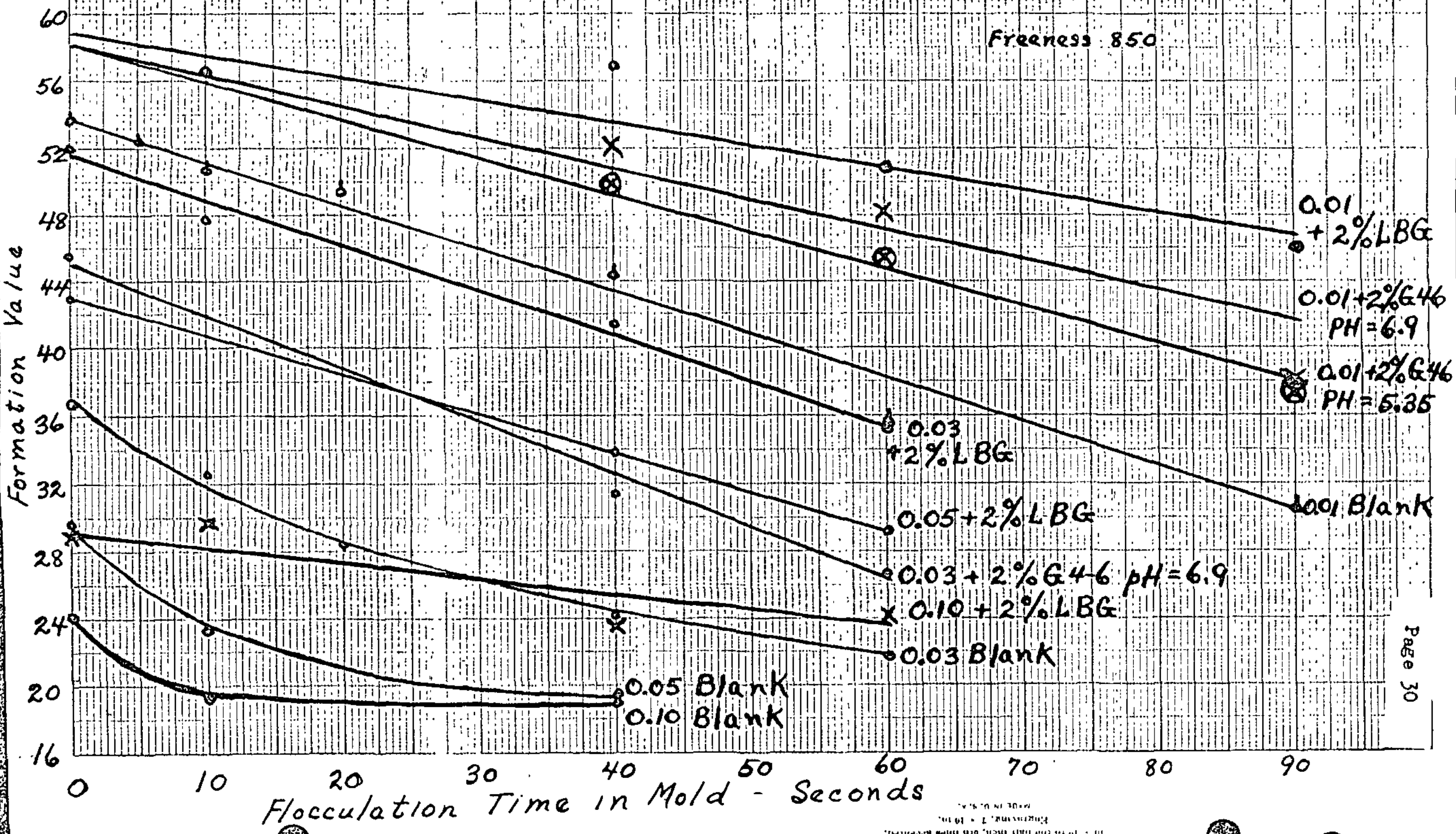
| IPC File No. | Mucilage Added | Consistency, % | Floccula-tion Time Seconds | THWING FORMATION VALUE Max. | Min. | Av. | Bursting Strength Points |
|---|---|---|---|---|---|---|---|
| 118004 | Blank | 0.01 | 0 | 54.9 | 52.1 | 53.6 | 12.5 |
| | | 0.01 | 5 | 54.3 | 50.5 | 52.3 | 15.5 |
| | | 0.01 | 10 | 51.5 | 50.5 | 50.8 | 15.7 |
| | | 0.01 | 20 | 50.0 | 49.0 | 49.5 | 16.3 |
| | | 0.01 | 40 | 44.6 | 43.5 | 44.2 | 16.8 |
| | | 0.01 | 60 | 35.7 | 35.2 | 35.5 | 15.9 |
| | | 0.01 | 90 | 31.6 | 28.7 | 30.3 | 14.1 |
| 118005 | Blank | 0.03 | 0 | 38.8 | 34.2 | 36.8 | 15.1 |
| | | 0.03 | 10 | 33.8 | 31.6 | 32.5 | 15.0 |
| | | 0.03 | 20 | 29.8 | 26.3 | 28.4 | 14.3 |
| | | 0.03 | 40 | 24.5 | 23.6 | 24.1 | 13.9 |
| | | 0.03 | 60 | 22.7 | 20.8 | 21.8 | 13.0 |
| 118006 | 2% LBG | 0.01 | 10 | 58.8 | 54.3 | 56.6 | 26.1 |
| | | 0.01 | 40 | 57.5 | 55.6 | 56.9 | 27.4 |
| | | 0.01 | 60 | 52.1 | 48.5 | 50.9 | 27.7 |
| | | 0.01 | 90 | 48.1 | 43.1 | 46.0 | 26.8 |
| 118007 | 2% C-46 | 0.01 | 40 | 50.5 | 47.6 | 49.0 | 22.7 |
| | pH = 5.35 | 0.01 | 60 | 48.1 | 41.7 | 45.2 | 23.5 |
| | | 0.01 | 90 | 37.9 | 36.0 | 36.8 | 24.1 |
| 118008 | Blank | 0.01 | 40 | 43.1 | 39.4 | 41.9 | 15.8 |
| | | 0.01 | 60 | 37.6 | 35.2 | 36.5 | 15.0 |
| 118009 | Blank | 0.05 | 0 | 31.6 | 28.4 | 29.8 | 13.7 |
| | | 0.05 | 10 | 23.6 | 22.7 | 23.3 | 13.1 |
| | | 0.05 | 40 | 20.0 | 18.4 | 19.4 | 11.6 |

TABLE X (Continued)

| IPC File No. | Mucilage Added | Consistency, % | Flocculation Time Seconds | THWING FORMATION VALUE Max. | Min. | Av. | Bursting Strength Points |
|---|---|---|---|---|---|---|---|
| 118010 | Blank | 0.01 | 40 | 44.6 | 41.0 | 42.8 | 16.0 |
| | | 0.01 | 60 | 37.3 | 35.7 | 36.5 | 15.2 |
| 118011 | Blank | 0.10 | 0 | 25.3 | 23.1 | 24.1 | 12.1 |
| | | 0.10 | 10 | 20.8 | 18.8 | 19.5 | 11.0 |
| | | 0.10 | 40 | 20.8 | 17.7 | 19.5 | 12.0 |
| 118012 | 2% G4-6 pH = 6.9 | 0.01 | 40 | 53.2 | 51.5 | 52.3 | 24.9 |
| | | 0.01 | 60 | 49.0 | 47.2 | 48.1 | 24.2 |
| | | 0.01 | 90 | 38.8 | 36.8 | 37.6 | 23.3 |
| 118013 | 2% G4-6 pH = 6.9 | 0.03 | 0 | 48.5 | 43.5 | 45.7 | 24.7 |
| | | 0.03 | 40 | 33.3 | 30.1 | 31.3 | 23.0 |
| | | 0.03 | 60 | 27.8 | 25.8 | 26.5 | 22.0 |
| 118014 | 2% LBG | 0.03 | 0 | 53.8 | 49.5 | 52.0 | 25.9 |
| | | 0.03 | 10 | 49.0 | 46.3 | 47.8 | 26.3 |
| | | 0.03 | 40 | 45.5 | 38.5 | 41.4 | 24.5 |
| | | 0.03 | 60 | 36.8 | 34.7 | 35.6 | 24.0 |
| 118015 | 2% LBG | 0.05 | 0 | 44.2 | 41.7 | 43.0 | 24.2 |
| | | 0.05 | 40 | 35.2 | 32.5 | 33.8 | 24.4 |
| | | 0.05 | 60 | 30.5 | 28.4 | 29.1 | 21.5 |
| 118016 | 2% LBG | 0.10 | 0 | 30.5 | 28.1 | 29.2 | 20.6 |
| | | 0.10 | 10 | 31.6 | 26.6 | 29.9 | 21.1 |
| | | 0.10 | 40 | 24.8 | 22.7 | 23.8 | 19.3 |
| | | 0.10 | 60 | 26.6 | 22.7 | 24.1 | 20.2 |

# Fig. 5

## Effect of Flocculation Time, Consistency, and Mucilage on the Formation Value of Handsheets



The above method appeared to be suitable for studying the effect of beating on the formation effect. The pulp was beaten to a freeness of 600 and sheets made at various consistencies with and without 2% locust bean gum. The data are in Table XI and Figure 6 wherefrom it appears that beating of the pulp in the Valley beater decreases the formation value.

In the experiments wherein the formation qualities of various mucilages were compared, it was noted that 4.5% of alum completely destroyed the deflocculating effect of the mucilage. It seemed desirable to obtain more data on this in the presence of rosin.

TABLE XI

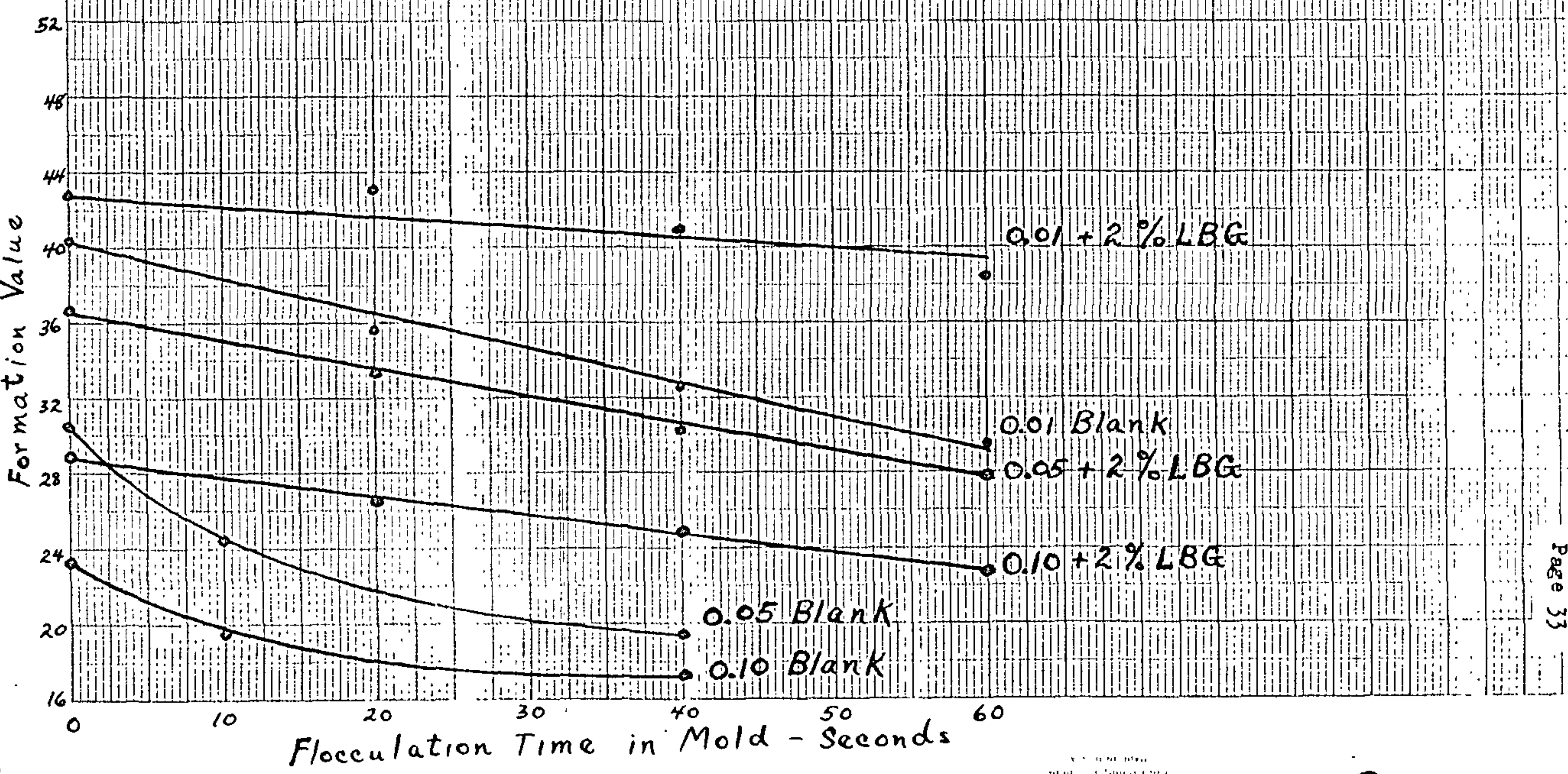
EFFECT OF TIME OF FLOCCULATION AND CONSISTENCY ON THE FORMATION VALUE OF HANDSHEETS
(Freeness 600)

| IPC File No. | Mucilage Added | Pulp Consistency, % | Flocculation Time Seconds | Thwing Formation Value Max. | Thwing Formation Value Min. | Thwing Formation Value Av. | Basis Weight 25x40/500 | Bursting Strength Points | Bursting Strength pt./100 lb. |
|---|---|---|---|---|---|---|---|---|---|
| 118154 | Blank | 0.01 | 0 | 43.1 | 37.6 | 40.2 | 46.8 | 43.0 | 92 |
| | Drain Time | 0.01 | 20 | 36.2 | 35.2 | 35.7 | 46.0 | 42.8 | 93 |
| | 14-15 seconds | 0.01 | 40 | 32.9 | 32.1 | 32.5 | 46.5 | 42.6 | 92 |
| | | 0.01 | 60 | 29.8 | 29.1 | 29.6 | 46.0 | 39.6 | 86 |
| 118155 | 2% LBG | 0.01 | 0 | 43.1 | 42.4 | 42.9 | 46.0 | 53.3 | 116 |
| | Drain time | 0.01 | 20 | 45.5 | 41.7 | 43.2 | 45.7 | 52.4 | 115 |
| | 17-18 seconds | 0.01 | 40 | 41.7 | 40.3 | 41.0 | 46.6 | 54.5 | 117 |
| | | 0.01 | 60 | 39.1 | 37.9 | 38.3 | 46.5 | 54.9 | 118 |
| 118156 | Blank | 0.05 | 0 | 31.3 | 29.8 | 30.4 | 45.1 | 37.9 | 84 |
| | Drain time | 0.05 | 10 | 24.8 | 24.0 | 24.4 | 45.8 | 36.8 | 80 |
| | 3 seconds | 0.05 | 40 | 19.7 | 19.4 | 19.5 | 45.2 | 34.2 | 76 |
| 118157 | 2% LBG | 0.05 | 0 | 37.3 | 36.2 | 36.6 | 45.2 | 48.3 | 107 |
| | Drain time | 0.05 | 20 | 33.8 | 32.1 | 33.1 | 45.3 | 49.7 | 110 |
| | 4 seconds | 0.05 | 40 | 31.3 | 29.1 | 30.1 | 45.8 | 48.9 | 107 |
| | | 0.05 | 60 | 28.4 | 27.5 | 27.9 | 45.9 | 48.7 | 106 |
| 118158 | Blank | 0.10 | 0 | 24.5 | 22.3 | 23.2 | 45.0 | 34.0 | 76 |
| | Drain time | 0.10 | 10 | 20.0 | 18.8 | 19.5 | 44.6 | 32.7 | 73 |
| | 2 seconds | 0.10 | 40 | 18.2 | 16.3 | 17.1 | 44.1 | 30.0 | 68 |
| 118159 | 2% LBG | 0.10 | 0 | 30.1 | 27.5 | 28.9 | 44.3 | 43.6 | 98 |
| | Drain time | 0.10 | 20 | 27.8 | 25.5 | 26.4 | 44.2 | 39.8 | 90 |
| | 2 seconds | 0.10 | 40 | 26.3 | 23.6 | 24.9 | 44.2 | 42.7 | 97 |
| | | 0.10 | 60 | 23.6 | 22.1 | 22.8 | 44.2 | 41.8 | 95 |

Fig. 6

Effect of Flocculation Time, Consistency, and Mucilage on the Formation Value of Handsheets

Freeness 600



A practically unbeaten stock was used being the same freeness as earlier experiments--about 850. Two per cent of rosin was added and various quantities of alum. The pH in the sheetmold was adjusted to about 5 each time with 3 N $H_2SO_4$. A consistency of 0.03% was used. The data are in Table XII and Figure 7. It appears under the conditions of these experiments that sufficient alum to give a desirable size test decreases the formation of the sheet but does not bring it anywhere near the blank sheet excepting in the presence of impractical excessive quantities.

The size test in the presence of mucilage ran about 30-40 seconds lower than when the mucilage was absent. This may be enough to seriously affect mill-made papers and should be watched for in future mill trials when larger quantities of mucilage are used.

TABLE XII

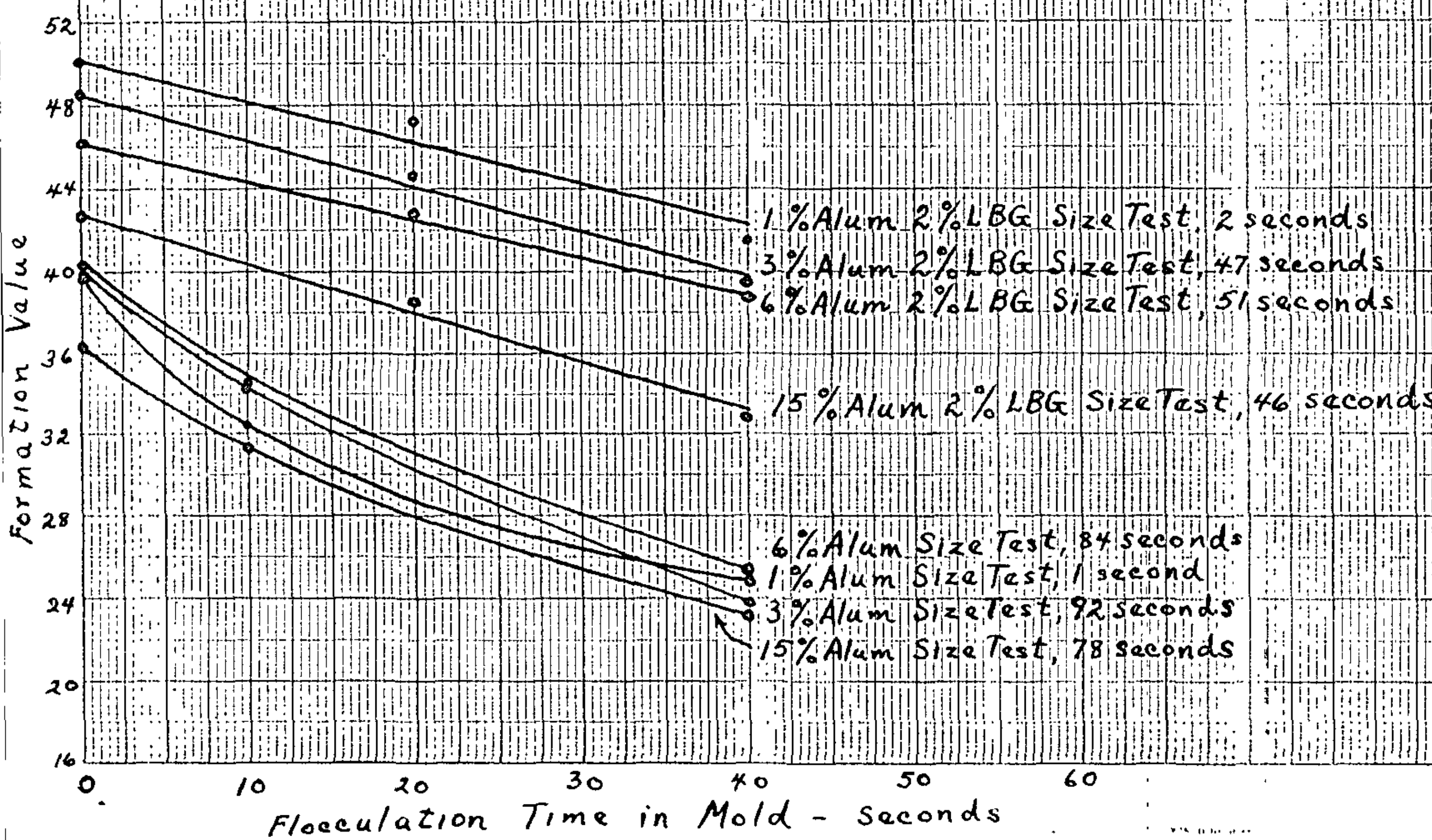
EFFECT OF ROSIN AND VARIOUS AMOUNTS OF ALUM ON THE FORMATION VALUE
(Freeness 850, Rosin 2%, Consistency 0.03%)

| IPC File No. | Mucilage Added | Alum ADded % | Floccula-tion Time Seconds | Basis Weight 25x40/500 | Bursting Strength Points | Bursting Strength pt./100 lb. | Valley Size Seconds | THWING FORMATION VALUE Max. | Min. | Av. |
|---|---|---|---|---|---|---|---|---|---|---|
| 118238 | Blank | 1 | 0 | 47.1 | 17.6 | 37 | 1 | 40.3 | 38.5 | 39.7 |
| | | 1 | 10 | 47.9 | 17.7 | 37 | 1 | 34.2 | 31.3 | 32.4 |
| | | 1 | 40 | 47.0 | 15.8 | 34 | 1 | 26.0 | 23.4 | 24.9 |
| 118239 | Blank | 3 | 0 | 48.8 | 19.5 | 40 | 93 | 40.3 | 39.7 | 39.9 |
| | | 3 | 10 | 48.4 | 18.7 | 39 | 96 | 35.2 | 33.3 | 34.2 |
| | | 3 | 40 | 48.0 | 17.2 | 36 | 86 | 24.8 | 22.7 | 23.9 |
| 118240 | Blank | 6 | 0 | 47.9 | 18.0 | 38 | 89 | 41.0 | 38.5 | 40.3 |
| | | 6 | 10 | 46.8 | 17.9 | 38 | 79 | 35.7 | 32.9 | 34.3 |
| | | 6 | 40 | 46.1 | 16.7 | 36 | 80 | 26.0 | 24.8 | 25.3 |
| 118244 | Blank | 15 | 0 | 47.7 | 17.3 | 36 | 76 | 36.8 | 35.7 | 36.2 |
| | | 15 | 10 | 49.0 | 17.9 | 37 | 96 | 32.5 | 30.9 | 31.4 |
| | | 15 | 40 | 47.7 | 16.8 | 35 | 64 | 24.3 | 22.7 | 23.5 |
| 118241 | 2% LBG | 1 | 0 | 47.4 | 31.1 | 66 | 2 | 52.1 | 49.0 | 50.0 |
| | | 1 | 20 | 46.5 | 29.8 | 64 | 2 | 48.1 | 46.3 | 47.2 |
| | | 1 | 40 | 47.4 | 30.8 | 65 | 2 | 43.1 | 39.7 | 41.3 |
| 118242 | 2% LBG | 3 | 0 | 47.1 | 29.6 | 63 | 46 | 50.0 | 46.3 | 48.4 |
| | | 3 | 20 | 46.6 | 30.2 | 65 | 44 | 46.3 | 42.4 | 44.7 |
| | | 3 | 40 | 46.8 | 29.2 | 62 | 52 | 40.3 | 38.5 | 39.5 |
| 118243 | 2% LBG | 6 | 0 | 47.0 | 29.6 | 63 | 49 | 47.2 | 45.5 | 46.1 |
| | | 6 | 20 | 47.1 | 30.1 | 64 | 54 | 43.1 | 42.4 | 42.9 |
| | | 6 | 40 | 47.2 | 28.1 | 60 | 53 | 39.1 | 38.5 | 38.9 |
| 118245 | 2% LBG | 15 | 0 | 45.1 | 27.9 | 62 | 47 | 44.6 | 39.7 | 42.7 |
| | | 15 | 20 | 45.6 | 28.1 | 62 | 47 | 40.3 | 35.7 | 38.6 |
| | | 15 | 40 | 44.9 | 26.5 | 59 | 44 | 35.2 | 31.3 | 32.9 |

# Fig. 7

## Effect of Rosin and Alum on Formation Value

Freeness 850, Rosin 2%, Consistency 0.03%



The bursting strength values of these sheets seem to indicate that the formation value affects this test to a smaller degree than expected.

SUGGESTIONS FOR FUTURE WORK:

1. The effects of rosin and alum on the formation value of a more highly beaten stock should be made.

2. Further attempts should be made to determine why the guar mucilages possess lower formation qualities than locust bean gum.

3. The new G4-5 mucilage should be evaluated.

jws/ejh

# PROJECT REPORT FORM

Copy to: Files
Mr. Steele
Dr. Rowland
Mr. Swanson

PROJECT NO. 849
COOPERATOR Institute
REPORT NO. 14
DATE July 11, 1945
NOTE BOOK 631
PAGE 22 TO 26
SIGNED John W. Swanson
John W. Swanson

## EXPERIMENTS ON THE EFFECTS OF COLLOIDAL FERRIC OXIDE, RESIDUAL BLEACH LIQUOR AND ROSIN AND ALUM ON THE RELATIVE POTENCY OF GUAR MUCILAGE

### Introduction:

On several occasions during mill trials of mannogalactan mucilages there have been indications that certain substances interfered with the action of the mucilage. This was noticed particularly during a series of trials at the Hoberg Paper Mills. Examination of their pulps revealed the presence of considerable quantities of iron and residual bleach liquor residues. Addition of larger quantities of mucilage to the pulp seemed to overcome the effects of the detrimental agent. It seemed desirable to investigate the possible effects of some of these foreign substances on laboratory beaten pulps. The following factors were included in the study.

1. The effect of using water containing 20 p.p.m. of colloidal ferric oxide.
2. The effect of using pulp containing 2% of residual bleach liquor.
3. The effect of rosin and alum.

### Experimental:

Preparation of Colloidal Ferric Oxide Sol.

A concentrated ferric chloride solution was made and poured into a large excess of boiling water. After cooling, the solution was diluted to one per cent iron concentration.

Beating of Pulp.

Three hundred ninety grams of Weyerhaeuser bleached sulfite were beaten at 1.5 per cent consistency in the Valley beater for 34 minutes with 4500 g. on the bed plate. Two such batches of pulp were made and mixed in a large 10 gallon crock. Schopper-Riegler Freeness was 750.

The pulp used for the experiments on the effects of residual bleach liquor was beaten in the same way except that 2% of bleach liquor containing 1.5% of available chlorine was added at the beginning of the beating cycle. Freeness about 700.

Cooking of Mucilage.

Two and one-half grams of G4-6 mucilage were added to 250 ml. of 0.25% borax solution and steam injected until the temperature reached 80° C. Hydrochloric acid was then added to bring the pH to about 5.0 - 5.5. After stirring at 80° C. for 10 minutes the mixture was diluted to 0.5% mucilage concentration, cooled and used.

Making of Sheets.

Forty grams of the beaten pulp (approximately 2680 ml. of 1.5% stock) were measured out and the desired quantity of G4-6 mucilage weighed out and added with vigorous stirring. After 5 minutes the stock was diluted to 0.5% consistency and eight 1.5 g. handsheets were made with an

8 count interval in the sheet mold prior to forming the sheet. Then 2% of rosin size (based on the remaining stock) was stirred in for 5 minutes followed by 4.5% of alum and another 5 minute stirring period. Eight more sheets were made with sufficient additional alum added to the sheet mold to bring the pH to about 5. before forming the sheet. In those cases where iron was desired in the pulp a sufficient volume of the colloidal iron solution to furnish 20 p.p.m. of iron to 8 liters was measured out and added before the mucilage. After five minutes the mucilage and remaining water were added as before. Iron was also added to the sheet mold prior to the formation of the sheet.

Results and Discussion.

The handsheet data are presented in Tables I to VI. It appears that iron, residual bleach liquor, and rosin and alum do not act detrimentally upon the mucilage under the conditions used in these experiments. The amount of iron used is considerably in excess of that found in usual paper mill waters. It is probable that the amount present in the pulp itself has a greater influence on the mucilage than that present in the water alone. For this reason the colloidal iron effects were exaggerated by adding the concentrated iron solution to the 1.5% pulp before dilution. Even this procedure did not seem to impair the mucilage action. It has been found in another series of experiments that colloidal ferric oxide present in pulp is distinctly detrimental to the formation improvement characteristics of mannogalactan mucilages. The handsheets of the present experiments were made in such a way as to promote good formation in all cases. It would appear, therefore, that the detrimental effect of iron in commercial pulps might be explained by the influence on the formation of the sheet since poorer formation results in lower strength values.

In all cases the use of rosin and alum decreased the strength of the resultant handsheets. This does not represent an impairment of the mucilage effects since the blanks were affected a like amount in strength. In other words, the use of rosin and alum decreased the bursting strength almost a constant number of mullen points throughout the cycle 0 to 1.5% G4-6 mucilage.

TABLE I

PROPERTIES OF HANDSHEETS CONTAINING G4-6 MUCILAGE

(No rosin or alum)

| Institute File No. | Per cent G4-5 Added | Basis Weight 25x40/500 | Caliper inch | Bursting Points | Strength Points/ 100 lbs. | Per cent Increase in Burst | MIT Fold | Per cent Increase in Fold | Porosity Sec./ 100 cc. | Valley Size Seconds | Tear g/ Sheet | Tear Factor |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 116698 | Blank | 46.4 | 0.0044 | 31.1 | 67 | — | 174 | — | 20 | 1 | 72 | 1.55 |
| 116700 | 0.1 | 46.8 | 0.0043 | 32.0 | 68 | 1.5 | 213 | 22.4 | 21 | 1 | 63 | 1.35 |
| 116702 | 0.25 | 46.3 | 0.0044 | 34.2 | 74 | 10.4 | 228 | 31.0 | 24 | 1 | 64 | 1.38 |
| 116704 | 0.5 | 47.3 | 0.0045 | 38.5 | 81 | 20.9 | 282 | 62.2 | 25 | 1 | 63 | 1.33 |
| 116706 | 1.5 | 47.6 | 0.0046 | 41.2 | 87 | 29.4 | 497 | 185. | 25 | 1 | 65 | 1.37 |

TABLE II

PROPERTIES OF HANDSHEETS CONTAINING G4-6 MUCILAGE AND 2% ROSIN SIZE AND 4.5% ALUM

| Institute File No. | Per cent G4-5 Added | Basis Weight 25x40/500 | Caliper inch | Bursting Points | Strength Points/ 100 lbs. | Per cent Increase in Burst | MIT Fold | Per cent Increase in Fold | Porosity Sec./ 100 cc. | Valley Size Seconds | Tear g/ Sheet | Tear Factor |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 116699 | Blank | 47.7 | 0.0045 | 28.5 | 60 | — | 77 | — | 19 | 29 | 75 | 1.57 |
| 116701 | 0.1 | 48.3 | 0.0046 | 30.5 | 63 | 5.0 | 102 | 32.5 | 20 | 28 | 68 | 1.41 |
| 116703 | 0.25 | 48.1 | 0.0046 | 33.5 | 70 | 16.7 | 175 | 127. | 21 | 23 | 73 | 1.52 |
| 116705 | 0.5 | 49.5 | 0.0047 | 36.5 | 74 | 23.4 | 262 | 240. | 21 | 30 | 69 | 1.39 |
| 116707 | 1.5 | 48.0 | 0.0046 | 39.6 | 83 | 38.4 | 298 | 288. | 18 | 27 | 62 | 1.29 |

TABLE III

PROPERTIES OF HANDSHEETS CONTAINING G4-6 MUCILAGE AND 20 P.P.M. OF COLLOIDAL FERRIC IRON

(No rosin or alum)

| Institute File No. | Per cent G4-5 Added | Basis Weight 25x40/500 | Caliper inch | Bursting Points | Strength Points/ 100 lbs. | Per cent Increase in Burst | MIT Fold | Per cent Increase in Fold | Porosity Sec./ 100 cc. | Valley Size Seconds | Tear g/ Sheet | Tear Factor |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 116708 | Blank | 45.8 | 0.0045 | 31.0 | 68 | — | 167 | — | 20 | 1 | 69 | 1.51 |
| 116710 | 0.1 | 45.8 | 0.0043 | 33.0 | 72 | 5.9 | 207 | 24.0 | 18 | 1 | 66 | 1.44 |
| 116712 | 0.25 | 46.9 | 0.0045 | 34.3 | 73 | 7.4 | 196 | 17.4 | 21 | 1 | 54 | 1.36 |
| 116714 | 0.5 | 45.0 | 0.0043 | 36.8 | 82 | 20.6 | 265 | 58.7 | 19 | 1 | 58 | 1.29 |
| 116716 | 1.5 | 46.8 | 0.0044 | 37.8 | 81 | 19.1 | 398 | 138. | 24 | 1 | 56 | 1.20 |

TABLE IV

PROPERTIES OF HANDSHEETS CONTAINING G4-6 MUCILAGE, 20 P.P.M. OF COLLOIDAL FERRIC IRON AND 2% ROSIN SIZE AND 4.5% ALUM

| Institute File No. | Per cent G4-5 Added | Basis Weight 25x40/500 | Caliper inch | Bursting Points | Strength Points/ 100 lbs. | Per cent Increase in Burst | MIT Fold | Per cent Increase in Fold | Porosity Sec./ 100 cc. | Valley Size Seconds | Tear g/ Sheet | Tear Factor |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 116709 | Blank | 47.0 | 0.0045 | 28.3 | 60 | — | 99 | — | 16 | 29 | 69 | 1.47 |
| 116711 | 0.1 | 46.0 | 0.0043 | 29.1 | 63 | 5.0 | 112 | 13.3 | 15 | 24 | 72 | 1.57 |
| 116713 | 0.25 | 49.5 | 0.0047 | 35.0 | 71 | 18.3 | 187 | 89.0 | 21 | 32 | 69 | 1.39 |
| 116715 | 0.5 | 46.9 | 0.0045 | 35.3 | 75 | 25.0 | 216 | 118.0 | 23 | 25 | 61 | 1.30 |
| 116717 | 1.5 | 48.3 | 0.0046 | 39.6 | 82 | 36.7 | 341 | 244.0 | 25 | 26 | 60 | 1.24 |

TABLE V

PROPERTIES OF HANDSHEETS MADE FROM PULP CONTAINING G4-6 MUCILAGE AND 2% OF RESIDUAL BLEACH LIQUOR

(No rosin or alum)

| Institute File No. | Per cent G4-5 Added | Basis Weight 25x40/500 | Caliper inch | Bursting Points | Strength Points/ 100 lbs. | Per cent Increase in Burst | MIT Fold | Per cent Increase in Fold | Porosity Sec./ 100 cc. | Valley Size Seconds | Tear g/ Sheet | Tear Factor |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 116718 | Blank | 46.2 | 0.0043 | 33.2 | 72 | — | 169 | — | 32 | 1 | 63 | 1.36 |
| 116720 | 0.1 | 46.9 | 0.0043 | 35.4 | 76 | 5.6 | 235 | 39.1 | 34 | 1 | 62 | 1.32 |
| 116722 | 0.25 | 46.4 | 0.0042 | 37.9 | 82 | 13.9 | 193 | 14.2 | 34 | 1 | 57 | 1.23 |
| 116724 | 1.5 | 45.7 | 0.0042 | 40.7 | 89 | 23.6 | 358 | 112. | 39 | 1 | 55 | 1.20 |

TABLE VI

PROPERTIES OF HANDSHEETS MADE FROM PULP CONTAINING, G4-5 MUCILAGE, 2% RESIDUAL BLEACH LIQUOR AND 2% ROSIN SIZE AND 4.5% ALUM

| Institute File No. | Per cent G4-5 Added | Basis Weight 25x40/500 | Caliper inch | Bursting Points | Strength Points/ 100 lbs. | Per cent Increase in Burst | MIT Fold | Per cent Increase in Fold | Porosity Sec./ 100 cc. | Valley Size Seconds | Tear g/ Sheet | Tear Factor |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 116719 | Blank | 46.8 | 0.0045 | 29.3 | 63 | — | 104 | — | 20 | 9 | 66 | 1.41 |
| 116721 | 0.1 | 48.3 | 0.0045 | 33.4 | 69 | 9.5 | 154 | 48.1 | 25 | 15 | 60 | 1.24 |
| 116723 | 0.25 | 47.1 | 0.0044 | 34.5 | 73 | 15.9 | 212 | 104. | 24 | 13 | 59 | 1.25 |
| 116725 | 1.5 | 48.8 | 0.0046 | 41.7 | 86 | 36.6 | 329 | 218. | 26 | 16 | 55 | 1.13 |