REPORT

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by George E. Holm for the degree of Master of Science They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science

R. W. Thatcher
Chairman

R A Gortner

Edward E. Nicholson

1918

THE SOLUBLE NON-SUGAR SOLIDS
OF SORGHUM JUICE

-o-

A thesis submitted to the Faculty of the Graduate School of the University of Minnesota

by

George E. Holm

in partial fulfillment of the requirements for the degree of Master of Science

June, 1916

TABLE OF CONTENTS

****

INTRODUCTION

There are present in sorghum and sugar cane juices compounds commonly called "non-sugars" or "non-sugar solids". These consist, so far as we know, mainly of organic acids and salts, acid amids, nitrogen bases, and mucilagenous and gummy substances commonly termed "gums".

These compounds, although present only in small proportions, are objectionable from the standpoint of the use of the juice for manufacture of sugar or syrup. The disadvantages arising from their presence in the juice are of two types. There is, first, the difficulties which they cause in the manufacturing processes; and, second, the undesirability of their presence in the food product after it is finished.

The difficulties which they introduce into the technical processes of sugar manufacture or syrup making are of two kinds. First, there are the errors which their presence causes in the results of analysis of the raw juice and of the various intermediate products in the process, making it difficult to properly control the various steps in the process. In the second place, the complete removal of these non-sugars from the juice, by the various clarification processes, is a difficult and expensive undertaking. If they are not completely removed, the final product may have unsatisfactory keeping qualities, or any undesirable flavor, ormay be unfit for food because of the presence of these impurities which may have some undesirable physiological action.

In the manufacture of syrups the amino acid amids are not removed in clarification, and during evaporation change to their corresponding amino acids giving off ammonia during the change. For this reason the acids predominate over the acid amids in the syrup more so than in the juice.[1]

As a result of various investigations Zerban states[2] "that the acid amids and amino acids found in the mercuric nitrate precipitate must undoubtedly be classed as objectionable nitrogen compounds in light of Prinsen Geerlig's theory of molasses." The error they produce in determination of sugars by polariscopic methods would depend entirely upon the predominance of laevo- over dextro-rotatory compounds or vice versa. Zerban found[1] that asparagin in the presence of lead subacetate had a specific rotatory power equal to sucrose, so that each percent asparagin would then produce an error of 1% in the polariscopic reading. Glutamin, however, has a laevo-rotatory power and partly neutralizes the error produced by asparagin. He therefore considered the error negligible except in abnormal cases.

The presence of certain nitrogen bases such as guanin, cholin, and betain would probably have no commercial significance due to the minute quantities that are present, but are of interest only from a physiological standpoint.

The "so-called gums", altho optically active, present a negligible error in determinations and can be completely removed by mercuric nitrate. In clarification in sugar manufacturing they are only partially removed and during the process of evaporation the remaining ones are partially hydrolyzed to their corresponding sugars. Since the remaining ones prevent crystallization of sugars, as shown by Maxwell,[3] this becomes the serious objection to their presence, if the sugar is to be recovered in crystallized form. In syrup manufacture this effect would not be a serious one.

HISTORICAL REVIEW

There have been no systematic studies of the nature or amount of soluble non-sugars in sorghum juice. But the fact that sorghum and sugar cane are closely related botanical species seemed to make it probable that the methods used and results obtained in similar studies on sugar cane might have a direct bearing upon the proposed studies with sorghum. On this account the literature dealing with the results of these investigations on sugar cane juices was reviewed, with the following results.

Considerable work has been done upon the nitrogenous compounds and also upon the "so-called gums" found in sugar cane juice.

Maxwell, at the Louisiana Experiment Station studied both the nitrogenous and nonnitrogenous substances.(3) He reported the presence of aspartic and glutaminic acids and (probably) the amids of these same acids. His conclusions were later confirmed by Zerban,(1) working at Rio Piedras, Porto Rico, who proved the presence of asparagin, glutamin and tyrosin and stated that other compounds were present in the mercuric nitrate precipitate.

Shorey,(4) working with Hawaiian cane juice, isolated guanin in small quantities and also found a lecithin(5) which on decomposition yielded cholin and betain. From the mercuric nitrate precipitate he isolated leucin and glycocoll.(6) Zerban, however, believes(2) that the substance isolated by Shorey was not glycocoll but an acid amid.

The proportional amounts of acids, acid amids and bases present in juices have been found to vary with the variety and age of the plant.[8] Amid nitrogen will decrease and albuminoid nitrogen increase as the plants grow older. The amount of any one of these present will therefore be a variable quantity. But work done by the above mentioned workers would give the following results as an average amount present in sugar cane juices at maturity.[2]

1. Nitrates 17.77 % of total nitrogen
2. Nitrogenous bases - very small - probably not over .005%
3. Amino acid nitrogen - 30.53% of total nitrogen
4. Amid nitrogen - 24.07% of total nitrogen.

The carbohydrate complexes such as polysaccharides, mucilages and gums, present an unsatisfactory phase of the investigations which have thus far been undertaken, since so little has been known of their exact chemical nature until recent years.

Maxwell[3] found that the "so-called gums" present in sugar cane juice consisted largely of pentosans (yielding pentose sugars on hydrolysis) and mucilages consisting of hexosans (or substances yielding hexose sugars on hydrolysis.)

Prinsen Geerlig[7] in his studies of bagasse reports 30% of cane gums in cane fibre, mostly xylan but possibly some araban.

The definite amount of each of these complex carbohydrates found in any case can not be accurately determined, and since they vary so much with different canes, seasons and methods of extraction, no average figures are to be obtained. Several experiment stations have, however, carried on series of experiments in clarification[1] and have reported the amounts present as percentage of "alcohol precipitate", since all of them are precipitated in a 50% alcohol solution.

The approximate amounts found have been as follows:

Juice	1st Mill (Extraction)	2nd Mill	Diffusion
Louisiana Cane	.96 %		70 %
" Purple Cane	.10%	.56%	
" " "	.24%	.62%	

This shows clearly the great difference in amounts extracted from different canes and by different methods.

PURPOSE AND SCOPE OF INVESTIGATION

The experiments which are briefly reviewed in the preceding paragraphs have all been conducted on sugar cane juice and in only a few instances have similar experiments been carried on with sorghum cane juices, and in no instances with sorghum grown in the northern states.

Because of the similarity in the plants and their products however, we may assume that their non-sugar solids might be similar both in character and amounts and sorghum juice may therefore contain any of the following:

1. Nitrogenous compounds such as acids, amids, nitrogen bases and coloring matters.
2. Non-nitrogenous compounds such as cellulose complexes, polysaccharides, and organic salts.

My work was undertaken with the idea and purpose of determining as nearly as possible, not only the nature of the substances present in sorghum cane juice, but also their approximate amounts. It was also thot probable that an idea of the compounds remaining in the juice after clarification might be gained thru the work.

It may be stated, however, that no work was done upon the coloring matters, nor was any special effort made to isolate non-nitrogenous organic salts and acids. The work was limited chiefly to the acids, amids, nitrogen bases and alcohol precipitate or "so-called gums."

EXPERIMENTAL WORK

(a) Securing and Preserving Samples.

For the preliminary experiments, fresh juice milled at this station was used. But because of the small proportions of non-sugars in the juice it was necessary to do most of the work with large quantities of material, and since suitable quantities could not be obtained in the immediate vicinity at convenient intervals, the juice was secured from a sorghum factory at Waconia, and was shipped in in one large lot.

This necessitated a method of preservation.

The juice was neutralized with sodium bicarbonate and saturated with toluol before being shipped. (This had been found, by West and Spriestersbach, working at this station, to be the best method of preserving juices for a long time.) On arrival it was frozen and placed in storage at a temperature of 14 degrees F. From time to time small lots of the frozen juice were taken for experiments performed.

During the progress of the work it was found that seepage of the heavier material had taken place and the juice at the bottom of the container was of a higher specific gravity than that nearer the top and consequently contained a higher percentage of nitrogenous and non-nitrogenous material. This fact must be taken into account in the interpretation of results obtained.

The above mentioned method of preservation may be objected to on the grounds that a "freeze" hydrolyzes the gummy substances or changes them into compounds that cannot be precipitated with alcohol.

It was found by a series of tests that such a change does occur but only to such a slight extent that for purposes in these investigations it is negligible. The gums of a frozen juice when precipitated with alcohol, not only have a lighter color but are less in amount, more flocculent and come down more slowly than does the precipitate of unfrozen juice. That they are very easily affected and changed even by low heat is shown by the following experiments:

(a) 2000 c.c. of a juice was condensed to 1100 c.c. in vacuuo at 40 degrees, p'pt'd by 1400 c.c. 95% alcohol, centrifuged, washed and dried and weighed, yielding 5.3840 gms.

(b) 2000 c.c. was precipitated with 2000 c.c. of 95% alcohol, centrifuged, washed, dried and weighed, yielding - 7.5485 gms.

With greater concentrations an equal volume of 95% alcohol will not complete the precipitation. In (a) this difficulty was experienced but the weight represents the total that would separate with alcohol as a precipitant. In (b), however, the precipitation was complete in an approximately 50% alcohol solution.

Despite the fact that they are easily changed the amounts yielded by frozen and unfrozen juices showed little variation and for our purpose the difference is as stated negligible.

500 c.c. juice precipitated by 600 c.c. 95% alcohol yielded 1.4180 gms.

500 c.c. frozen juice " " " " " yielded - 1.3940 "

(b) INVESTIGATION OF THE GUMS

(1) Precipitation of "Gums:" Several methods of precipitation have been suggested for gummy sbustances. It was thot that a better method of precipitation might be obtained than the "alcohol precipitation method," since this method was slow and difficult both in completeness of precipitation and handling of the precipitate, as experienced in former experiments. The following methods were tried - with 95% alcohol, 95% alcohol saturated with lead acetate, and alcohol acidified with HCl. The quantity of precipitate obtained in each case and its ash content are shown in the following table:

Precipitation	Wt. of pp't.	percent ash	Volatile and nonvolatile
A.[10] 1000 c.c. juice plus alcohol saturated with lead acetate	9.5920	48.67	
B. 2000 c.c. juice plus 2000 c.c. alcohol	7.5485	81.37	
C.[11] 500 c.c. juice plus alcohol slightly acidified with HCl (5 c.c.)	.7315	76.84	
D. 500 c.c. frozen juice plus alcohol and lead acetate	4.018	49.20	1.9768 nonvol. 2.0412 vol.
E. 500 c.c. frozen juice plus 600 c.c. alcohol	1.3940	-----	1.065 nonvol. .329 vol.

In an acid solution the gummy substances are very soluble, as shown in (c). The amount decreased to nearly one half despite the low acidity and short time allowed for settling (20 min.). From the standpoint of ease in handling the precipitate etc., the "alcohol-lead acetate" method was found to be the most convenient, but the amounts of volatile and nonvolatile portions as compared with (c) indicates that a large amount of other organic compounds are thrown down which might prove undesirable in further work on the gummy material.

The "alcohol precipitation" method was therefore used in all work on this phase of the problem.

(2) Amounts of "gums": Portions of juice from the top and bottom of the container showed different specific gravities and a corresponding difference in "alcoholic precipitate" content, as follows:

	Sample used	Sp. Gr.	Am't pp't.	percent on total
(a)	500 c.c. from top of container	1.013	1.3940	.260
(b)	500 c.c. from bottom of container	1.078	4.3000	.399

(b) is of the average density of sorghum juices and gives a fair estimate of the actual amount of "alcoholic precipitate" present and its composition. Of the 4.3 gms., precipitated in (b) the nitrogen compounds estimated as protein (N x 6) was found to be 12% of the precipitate and the ash 22.22%. This indicates that 65.28% is mucilages, gums and various other non-sugars.

For the purpose of determining more accurately the exact nature of the precipitate, 5 grams was boiled for 15 minutes in 300 c.c. of water, centrifuged, dried and weighed, with the following results:

Soluble portion Pp'td in 50% alcohol	1.3200 gms.	or 26.40% of total
Soluble portion not ppt'd in 50% alcohol	2.4863 "	or 49.73 " "
Insoluble portion	1.1937 gms.	or 23.87% " "

To sum up, the results are as follows:

1000 c.c. juice of specific gravity	1.078	
Total "Alcohol Precipitate"	4.30 gms.	
Percent " "	0.399%	
Composition of Precipitate		
Proteins		12.00%
Ash		22.22%
Gums, mucilages, etc.		65.28%
Solubility of Precipitate		
Insoluble in boiling water		23.87%
Soluble in boiling water, ppt'd by 50% alcohol		26.40%
Soluble in boiling water, not ppt'd by 50% alcohol		49.73%

This shows that the alcohol precipitate is made up mostly of soluble compounds and not over 25% of insoluble cellulose complexes and gums.

As compared with results on sugar cane juice these percentages in sorghum juice range higher in"alcohol precipitate," lower in ash and higher in soluble matter. The following table gives the comparisons:

	Sorghum Cane Juices	Sugar Cane Juices[9]
Soluble in hot water	approx. 65%	33% approx.
Insoluble in hot water	34%	33% (in 1% H_2SO_4 sol)
Alcohol precipitate	.399%	.181%
" "	.308%	.212%

(3) Investigation of Nature of "Gums": To determine the actual nature of the gummy bodies it is necessary to identify the sugars yielded by them on hydrolysis of both the soluble and insoluble

portions. Other microchemical tests and color reactions serve only to give an indication of what might be present.

The precipitate gave no blue or brown coloration with iodine, indicating the absence of starches and probably also of dextrins. Methylene blue stained numerous particles which di-colorized readily on addition of alcohol or dilute acids. This indicated a possibility of the presence of pectins.[12] To verify this a quantity of the soluble precipitate was reprecipitated in a 50% alcohol solution, dissolved in water and the jelly test performed as described by Goldthwaite.[13] Negative results were obtained, proving either the absence of pectins or that if present they are very minute in quantity; hence the coloration must be due to lignified or suberized cell walls.

A quantity of soluble precipitate was hydrolyzed for 15 minutes with dilute H_2SO_4.(1:5) The acid was removed with $Ba(OH)_2$ and the filtrate tested for xylose, arabinose and galactose. At the same time a few grams of insoluble precipitate was hydrolyzed with dilute H_2SO_4 for 20 hours and filtered while hot. A large mass of crystals resembling prolin separated on standing. These were evidently hydrolysis products of the coagulable proteins and were filtered off. Similar tests were then applied to this filtrate as those which were performed on the hydrolyzed soluble portion.

For identification of arabinose, galactose and xylose the osazones were formed and compared with osazones formed from the pure sugars. The "mucic acid test"[14] was also used for galactose. For xylose the "Bertrand reaction"[15] was used since this gives very characteristic, difficultly soluble, needle-shaped crystals on addition of Bromine and $CdCO_3$ to a solution of xylose.

(Note: No Di-phenyl-, Bromphenyl- or Methyphenyl-hydrazine was to be obtained to make the characteristic test for arabinose[16])

Following are the results of the tests:

Test	Soluble portion	Insoluble portion
Bertrand test for xylose	-	+
Osazones	+ for galactose — " arabinose	— for galactose — " arabinose
Mucic acid test	+	-

The strong positive test for galactose indicates the presence of mucilages or the closely allied substances, galactosans, which on hydrolysis yield galactose. Since galactose is found in the soluble portion and most true mucilages merely swell up in water it seems to indicate that the greater part of the substances are galactosans or other soluble complexes yielding galactose on hydrolysis. In the insoluble portion we find xylose, showing that xylan makes up the greater part of the insoluble precipitate. The unhydrolyzed quantity was washed and treated with Schweitzer's reagent but only a portion dissolved, indicating that the unhydrolyzed substance is made up partly of cellulose and partly of its insoluble complexes. Araban, whose presence in sugar cane juice has been doubted by some investigators and reported by others could not be detected in sorghum cane juice.

The "so-called gums" or alcohol precipitate of the sorghum juice investigated are therefore composed chiefly of galactosans, xylan, cellulose complexes, and possibly small quantities of mucilages and pectins.

(c) INVESTIGATION OF THE NITROGENOUS COMPOUNDS

(1) Amounts of Nitrogenous Compounds: The remaining non-sugars are chiefly made up of non-nitrogenous organic acids and salts, and nitrogenous compounds of various kinds. As heretofore stated the nitrogen content varies with the concentration of the juice and hence it was impossible to gain any figures for the average total amount. An average of the determinations made upon the least and the most concentrated juices should give an approximation for the average juice.

Total nitrogen determined by the Kjeldahl-Gunning method gave the following:

Juice of sp. gr. 1.013 gave	.0204%	of total nitrogen
" " " " 1.078 "	.0814%	" " "
Average -	.0509	" " "

This is slightly higher than the results obtained for other sorghum juices analyzed at this station but compare favorably with the average of sugar cane juices analyzed at the Louisiana station.[17]

To determine the nature of the nitrogen compounds present, two methods were used.

Method I: 50 c.c. of juice was precipitated by Stutzer's reagent and albuminoid and amid nitrogen determined. Total nitrogen was also determined on 50 c.c. The results are shown in the following table:

	Total Nitrogen	Albuminoid N. percent of total	Amid N percent of total
Sample 1	.0204	39.24	60.76
Sample 2	.0191	35.80	64.20

In case of sugar cane juice albuminoid nitrogen ranges from 25 to 50% of the total, varying with the age of the cane and also with the variety. Since the variation is so great, the figures for albuminoid and amid nitrogen give a means for comparison of nitrogen content of canes at different stages of growth and in comparison of varieties, and have been used extensively by Browne(1) and Maxwell(8) for this purpose.

Another method suggested itself to the writer as being more efficient in giving the actual nature of the nitrogen distribution in a quantitative manner.

Method II: A "Hausmann separation" was made on 150 c.c. of juice as suggested by D. D. Van Slyke(18). The following results not only give an insight into the nature of the nitrogen compounds but show that the sorghum plant juice is very similar to juices of other plants in amids, acids and bases.

Total Nitrogen	Amid Nitrogen	Proteins and bases ppt'd by phosphotungstic acid	Nitrogen not ppt'd by phosphotungstic acid
.0226%	.00508%	.00536%	.01216%
	22.47% of total	23.71% of total	53.82% of total

As noted the separation was carried out with a juice of low total nitrogen content. While we have no positive evidence that the same distribution of nitrogen-containing compounds would be found in juices of other concentrations, the assumption is that concentration does not effect these proportional amounts.

(2) Methods of Separation of Nitrogenous Compounds: A separation of nitrogen compounds is not complete with any one reagent. Lead acetate and lead subacetate are commonly used for

the separation of acids, but neither will precipitate the amids. The common precipitant for the latter is mercuric nitrate. Lead acetate alone was found to precipitate about one fourth of the total nitrogen from the juice, while lead subacetate precipitated slightly more. Mercuric nitrate added to the lead acetate filtrate threw down an additional amount about equal to that which the lead acetate had precipitated. The two reagents precipitated 51.53% of the total nitrogen. Since lead subacetate is a more complete precipitant than lead acetate it was added to the lead acetate mercuric nitrate filtrate in excess and a heavy precipitate settled out which contained about one fourth of the total nitrogen. This precipitate was evidently due to a double compound of mercury and lead since lead subacetate alone precipitated only 5.48% more than did lead acetate. This fractional precipitation method was therefore used with the following results:

Total Nitrogen percent	Precipitant	Nitrogen percent	Percent of total	
	Lead acetate	.0071	27.30	
	Filtrate $HgNO_3$	.0063	24.23	75.37
.0265	Filtrate lead subacetate	.0062	23.84	
	Filtrate	.0065	24.52	
		.0261	99.89	

In this manner it was possible to make three separations amounting to 75.37% of the total nitrogen present in the juice; while any one reagent, or both lead acetate and mercuric nitrate would yield not more than 51.53% of the nitrogen.

Forty-eight liters of juice was precipitated with lead acetate, the precipitate separated by decantation and centrifuging and washed free from sugars. The filtrate was precipitated with mercuric nitrate and the precipitate separated in the same manner as the first. To the lead acetate-mercuric nitrate filtrate was added lead subacetate in excess and the resulting precipitate filtered off and washed. The precipitate in each case was decomposed with H_2S, the metallic sulphide filtered off and the filtrate evaporated down under diminished pressure at a temperature of 30 degrees.

(3) Identification of Nitrogenous Compounds in each Fraction: Each portion was evaporated to a thin syrup and allowed to stand for some time. Brilliant octahedral crystals separated out from all portions in comparatively large quantities. The crystals were filtered off and washed but not purified. Upon ignition the crystals produced a white ash which proved to be calcium. From the insolubility and the shape of the crystals they were identified as calcium oxalate. .05 grams ignited produced .0335 grams volatile matter as compared with .0308, theoretical for calcium oxalate. This also indicates that the substance is calcium oxalate - the high value for volatile material being due to impurities.

The mother liquors from these crystals were evaporated to a very thick syrup in order to get rid of the acetic and nitric acids formed in the decomposition of the precipitates. A slight amount of water was added and the residues allowed to stand. From all residues a small amount of brilliant ball-shaped crystals were deposited. These were filtered off and recrystallized three times. They produced no ash on ignition proving them to be organic compounds. On addition of large quantities of water they went into solution at boiling temperature, producing a brown solution. The melting point could not be determined since they turned dark in color and evidently decomposed at from 286 - 288 degrees. The form of the crystals is characteristic of only one of the nitrogenous acids, amids or bases, namely "impure leucine"[20] which is classified by Abderhalden as l-leucin,[19] and well described by Hawkes.[20] In solubility and temperature of decomposition they agree with the compound described by Abderhalden, which decomposed at 293 - 295 degrees. The compound is undoubtedly "impure leucine" and identical with the compound isolated by Shorey[6] from Hawaiian cane juices.

The residue from the lead acetate fraction was again concentrated in vacuuo and allowed to stand. Minute quantities of crystals separated, which were composed of two characteristic types interspersed with small irregular crystals of various kinds. The crystals of one type were long and prism-shaped while those of the other were flat and hexagonal. Both were almost insoluble in water, insoluble in alcohol and dilute acetic acid solutions, but soluble in a sodium hydroxide solution. They were too minute in amount to make any quantitative tests upon them and only qualitative tests were applied. To a water solution of the crystals copper acetate was added but no precipitate formed. The remaining crystals were dissolved in a dilute sodium hydroxide solution and to this was added a solution of lead acetate. Upon standing the precipitate darkened showing that a sulphide was being formed and that the characteristic hexagonal prisms were undoubtedly cystin. Altho no conclusive test was obtained from the water solution and copper acetate it seems probable, from the properties exhibited and the reaction with a lead salt, that these crystals were aspartic acid and cystine interspersed with crystals of other amino acids.

To determine if there were any free amino acids left in the residue, it was divided into two portions. One was saturated with hydrogen chloride gas and to the other was added copper acetate and the solution heated. No precipitate was formed in either case, proving the absence of any free amino acids.

The residues from the mercuric nitrate and lead subacetate fractions were allowed to stand for a long time after being concentrated under diminished pressure. Large wedge-shaped crystals separated from both solutions in approximately equal amounts. These were slightly soluble in water and insoluble in alcohol and ether and decomposed at 207 - 210 degrees. (d-l-Asparagin 213 E. Abderhalden). They were acid in reaction and liberated ammonia when warmed with a 50% potassium hydroxide solution, proving that the compound is an amino acid amid. The pyrrol test[21] gave positive results. These tests prove that the compound is d-l-asparagin.

The filtrates were completely neutralized with calcium carbonate, filtered and again concentrated. In the solution from the mercuric nitrate fraction needle-shaped crystals, interspersed with asparagin, formed. These were probably identical with the glutamin needles separated from the asparagin filtrate by Zerban, working with the mercuric nitrate fraction of sugar cane juice.[2] The amount was, however, too small to permit isolation and identification.

SUMMARY

The soluble non-sugars of sorghum juice are present only in very small amounts, and their isolation and identification is, therefore, a matter of considerable difficulty.

The isolation of these compounds is made still more difficult by the fact that methods for the quantitative separation of many of the gummy carbohydrates and the gelatinous nitrogenous compounds have not yet been perfected.

Working with the best methods which are now known, and using large quantities of juice (forty-eight litres) the following compounds were isolated and identified:

(a) Non-nitrogenous Substances

1. Xylan
2. Galactans
3. Mucilages and pectins, (?), in small quantities
4. Cellulose and Insoluble Cellulose Complexes
5. Calcium oxalate

(b) Nitrogen-containing Compounds

1. Proteins
2. Leucine
3. Cystine, and possibly other amino acids
4. Asparagin

It is probable that there are other organic acids and salts, and perhaps also other nitrogenous compounds and carbohydrates than those here listed, present in sorghum juice; but the quantity present is so small that the time available for this work did not permit a search for them, using the very large quantities of

juice which would be necessary in order to be able to secure sufficient material to permit of its identification.

REFERENCES

1. Browne, C. A. "Clarification of Sugar Cane Juice" Louisiana Exp. Station Bul. 91:33 (1907).

2. Zerban, Fritz "Objectionable Nitrogenous Compounds in Sugar Cane Juice" 8th International Congress of Applied Chemistry, Vol. VIII Sec. Va (1912)

3. Maxwell, W. "Organic Solids not Sugars in Cane Juices" Bul. of the Louisiana Sugar Exp. Station Second Series, No. 38, 1895.

4. Shorey, E. C. "Xanthine Bases in Sugar Cane" Journal Am. Chem. Society, Vol. 21:609, (1899)

5. Shorey, E. C. "The Lecithins of Sugar Cane" Journal Am. Chem. Society, Vol. 19:881 (1897)

6. Shorey, E. C. "Additional Notes on the Sugar Cane Amid" Journal Am. Chem. Society, Vol. 20:133 (1898)

7. Geerlig, Prinsen Sugar Cane , 30:91.

8. Maxwell, W. "Organic Solids not Sugars in Cane Juices" Part I, Bul. of the Louisiana Sugar Exp. Station, Sec. Series No. 38, (1895)

9. Maxwell, W. "Organic Solids not Sugars in Cane Juices" Part II, Bul. of the Louisiana Sugar Exp. Station, Sec. Series No. 38, (1895)

10. Chauvin, A. C. "Moniteur scientifique" I:1, 317, (1911)

11. Chauvin, A. C. "Aunales des Falsifications" 5:27-30,(1911)

12. Hass, P. and Hill,T. Introduction to the Chemistry of Plant Products, Chap. II, p. 93-150.

13. Goldthwaite, N. E. "Contribution on the Chemistry and Physics of Jelly Making" Journal of Industrial and Engineering Chemistry Vol. 1:333, 1909.

14. Hawk, P. B. Practical Physiological Chemistry, 4th Edition, p. 41.

15. Abderhalden, E. Handbuch für Biochemischen Arbeitsmethoden, II:101.

16. Abderhalden, E. Handbuch für Biochemischen Arbeitsmethoden, II:100.

17. Browne, C. A. Jr. and Blonin, R. E. Louisiana Exp. Station Bulletin, 91:5 (1907.)

18. Van Slyke, D. D. "The Analysis of Proteins by Determination of the Chemical Groups Characteristic of the Different Amino Acids" Journal of Biological Chemistry, Vol. 10 pp. 15-55. (1911)

19. Abderhalden, E. Biochemisches Handlexikon, Band 4 p.559

20. Hawk, P. B. Practical Physiological Chemistry, 4th Edition, p. 367

21. Rosenthaler, L. Der Nachweis Organischer Verbindungen Band 19 and 20, p. 501 and 502.