A Study of the Carbohydrates of the Prickly Pear and Its Fruits.

DISSERTATION.

Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science of Columbia University . . . . . .

By

R. F. HARE, B. S., M. S.

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Publications

The following bulletins of the New Mexico Agricultural Experiment Station:—

No. 49, December, 1903. Canaigre, by R. F. Hare.

No. 60, November, 1906. Prickly Pear and Other Cacti as Food for Stock, by David Griffiths and R. F. Hare.

No. 64, April, 1907. The Tuna as a Food for Man, by R. F. Hare and David Griffiths.

No. 69, September, 1908. Experiments on the Digestibility of Prickly Pear by Cattle, by R. F. Hare.

No. 72, August, 1909. Denatured Alcohol from Tunas and Other Sources, by R. F. Hare, S. R. Mitchell, and A. P.Bjerregarrd.

Annual Reports of the Chemist, New Mexico Agricultural Experiment Station, from 1903 to 1911 inclusive.

The following bulletins of the United States Department of Agriculture:—

No. 106, March, 1909, Bureau of Animal Industry.

No. 102, September, 1907, Bureau of Plant Industry.

No. 116, December, 1907, Bureau of Plant Industry.

The following articles in the Journal of Industrial and Engineering Chemistry:

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Preliminary Study of the Carbohydrates in the Prickly Pear and Its Fruits

For several years, the Bureau of Plant Industry of the United States Department of Agriculture, and the Agricultural Experiment Station of New Mexico have conducted investigations to determine what value the cacti have as food for stock, the value of their fruits as food for man, as well as the possible utility of both the plant and its fruits for the several purposes that have been suggested by their nature and composition. In Bul. No. 60 of the New Mexico Experiment Station is given fodder analysis of one hundred and eighty-seven samples of cacti, and twenty-six complete ash analyses of these plants. These analyses together with the results of feeding experiments reported in Buls. No. 74 of the Bureau of Plant Industry and No. 91 of the Bureau of Animal Industry conclusively demonstrate the forage value of cacti.

In Buls. No. 64 of the New Mexico Experiment Station and No. 116 of the Bureau of Plant Industry are reported the results of a study of the value of the "tunas" (fruits of the prickly pear) as food for man. The nutritive value of the tuna is found to compare favorably with other American fruits, and explains clearly why the Mexicans make this fruit and its products a large part of their daily diet.

Buls. No. 69 of the New Mexico Experiment Station and No. 106 of the Bureau of Animal Industry give the results of some experiments made to determine the digestibility of prickly pear by cattle. In Bul. No. 72 of the New Mexico Experiment Station are recorded the results of a study of the possible utility of the tunas for the production of alcohol.

Since the cacti can be grown with little rainfall on the semi-arid plains of the Southwest where other useful vegetation will not thrive, an effort has been made to utilize this plant and its fruits in different ways, and many requests are received by the experiment Stations in the Southwest and by the United States Department of Agriculture for information relative to their possible utility. The prickly pear is now utilized very largely in the Southwest as food for stock, and for some localities its use has been highly recommended by the Bureau of Plant Industry as a farm crop. The fruits are used as food for man and beast. The Mexicans prepare preserves, beverages, and other edible products from the fruits, which also bid fair some day to be a valuable source of carbohydrate for the production of denatured alcohol. The fruits of certain varieties, such as *Opuntia dulcis* contain large quantities of a rich beautiful red pigment that is very useful in coloring candies, wines, ice cream, and other food products and drinks, where a vegetable color is preferred to coal tar dyes.

The nutritive value of the fruits depends almost entirely upon the quantity of sugar they contain. Sugar is present in the different edible species in amounts varying from seven to fifteen per cent, and is the only nutrient present in any appreciable quantity. The analyses of the fruits given in Bul. No. 64, referred to above, shows the sugars to be largely monoses or reducing sugars. A study of these tables reveals the rather interesting fact that, although very small amounts of sucrose are present, the juices of the fruits are often dextro-rotatory both before and after inversion. This is somewhat unusual for fruit sugars, which as a rule are levo-rotatory.

Composition of the Mucilage.

A fodder analysis of the stems of the prickly pear, made according to the conventional methods of the Association of Official Agricultural Chemists, shows this plant to contain 82.24 per cent of water, 3.03 per cent of ash, 12.54 per cent of organic matter, and 8.95 per cent of nitrogen-free extract. This last named material constitutes 71.3 per cent of the total organic matter, which shows

that the dried plant is composed largely of this kind of material. Such materials are often called carbohydrates, but since they merely represent that part of the air-dried, ether-extracted plant that is dissolved by consecutive treatments for thirty minutes each in 1.25 per cent solutions of sulphuric acid and caustic soda, it is very evident that many other substances may be present besides true carbohydrates. In the case of the prickly pear a large part of such an extract consists of mucilage. Some knowledge of the composition of this mucilage is very essential to a better understanding of its functions in this plant, as well as its importance in the various economic uses to which it might be applied. It is also valuable for an intelligent interpretation of the nutritive value of this plant, and to assist in determining the utility of the prickly pear in the production of alcohol, for which its employment has often been suggested. If, for example, this mucilage be composed of carbohydrates that hydrolyze to galactose and pentose, it can have little value for alcohol production, since pentoses are unfermentable and galactose ferments with difficulty.

It has been observed that the fruits of the prickly pear, when green, are, like the stem, filled with mucilage. During the ripening period this mucilage disappears from the fruits with a simultaneous production of sugar. In one instance a sample collected May 24th, just at the beginning of the ripening period, contained 4.0 per cent of reducing sugars, and was decidedly mucilagenous. On September 17th, fruits from the same plants had 11.92 per cent of sugar, and were apparently free from mucilage. These results indicate a possible change in the fruits from mucilage to sugar. If such a transformation does result, a logical explanation would be the simple hydrolysis of a polysaccharide into monoses. A study of the character of the fruit sugars shows that they are composed largely of glucose and fructose, with small amounts of sucrose, and perhaps a pentose in small amounts, but no galactose. Analysis of the carbohydrates of the stems, on the contrary, reveals the rather interesting fact that this mucilage contains galactan and a pentosan. If then there be a change from mucilage to sugar pro-

duced by the hydrolytic action of enzymes in the ripening fruits, it is quite evident that the transformation is more than one of simple hydrolysis. The carbohydrates of the stems are chiefly mucilage, with small amounts of gums, sugar and starch. The last named substance is present in considerable quantity during the summer months, but at other times it is quite scarce, if present at all.

Mucilages and Gums.

Mucilages and gums are substances which occur in solution in the cell sap of certain parts of a number of plants. When such sap exudes and dries on the surface of the plant the dessicated mass is generally called gum. The word gum is also sometimes used to designate substances in the plant cells which differ from mucilage in properties if not in composition. The function of this class of carbohydrates is not well known. They serve to protect the plant in the case of wounds, to enable them to retain water in times of drought, and are doubtless a form in which some plants store a surplus of food. Their composition is also not well understood, and has been little studied. This is doubtless due to the difficulty experienced in separating them from other sap substances like resins, starch, sugars, acids and inorganic constituents with which they are always associated in the plant. Mucilages are generally considered to be polysaccharides of different sugars. K. Yoshimura* investigated the character of the sugars present in the mucilage of several plants, and found that in some cases their hydrolysis resulted in the production of glucose, while in others galactose, arabinose or mannose were formed. Recently O'Sullivan has found that mucilages and gums are not polysaccharides, but glucoside derivatives of certain organic acids, the acid being different for each mucilage. So far as the Opuntias are concerned this view seems to be the correct one, since the amounts of sugars and ash are not sufficient to account for the total solids, and the residue from hydrolysis contains an undetermined acid.

Mucilages are undoubtedly complex substances that differ markedly in different plants, and apparently also in the same plant.

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*Yoshimura: Bul. Coll. of Agr. Imp. Uuniv., Tokyo, 1895, 2, 207–8.

For technical convenience a simple classification of mucilages is sometimes made into the following:

1. Those soluble in water, like gum arabic.
2. Those forming jellies in weakly acid solution, like pectin.
3. Those swelling in, but not dissolved by, water; like gum tragacanth.

Mucilages may be precipated with alcohol, are not fermented by yeast, do not reduce Fehling solution, and often yield furfural and mucic acid when heated with acids. These properties serve in part to distinguish them from the sap constituents with which they are associated. The prickly pear mucilage more nearly resembles the type that swells up, but is not soluble in water. It is apparently in perfect solution in the cell sap and when water is added it is thinned to a homogeneous mass, but if concentration of the sap is attempted the mucilage separates into thick and thin portions. If water is added to the air dry powdered plant it does not form a homogenous solution of the mucilage, but separates into thick and thin portions. When precipitated by alcohol the mucilage partly swells in water, but does not completely dissolve. The precipitate formed with two volumes of alcohol is grayish-white in color, and when stirred, collects in a stringy mass on the stirring rod. The filtrate is not mucilaginous, but when three more volumes of alcohol are added, a white flocculent precipitate is produced which is quite different from the first precipitate, both in appearance and composition. The first precipitate has a high content of pentosans, while the latter is free from this polysaccharide, but readily hydrolyzes to hexoses.

If the filtrate from the second precipitate is evaporated to a syrup and six to eight volumes of alcohol added, a white flocculent precipitate is formed that changes on drying to a golden yellow syrup.

Dilute solutions of the mucilage pass through filter paper, but when filtered through unglazed porcelain no mucilage passes into the filtrate, which seems to show that a true solution is not formed. While a one per cent extract is extremely viscous, its adhes-

ive properties are very poor. Experiments made by the Arthur D. Little Company to determine the utility of prickly pear mucilage for glazing paper showed it had little vaue for this purpose, because of its lack of adhesiveness. Dilute solutions of the mucilage do not rotate polarized light. The water solution is hydrolized by boiling water, by dilute acids or alkalies, and by distase. A one per cent solution of caustic soda dissolves the mucilage, and, since the immediate neutralization of such solutions with acid fails to restore the mucilaginous condition, it is evident the mucilage undergoes hydrolysis as a result of such treatment. When the alkaline solution of mucilage is precipitated with alcohol the precipitate is curdy and similar to that produced by alcohol from a solution after acid hydrolysis. One per cent solution of sodium carbonate does not change the charater of the mucilaginous solution even after heating for some time on the water bath.

A sample of the mucilage was hydrolyzed by treatment for several hours with boiling 1.25 per cent sulphuric acid solution. The acid was neutralized with barium carbonate, and the sugar converted into an osazone. This was readily soluble in hot water, had the characteristic orange yellow color of arabinosazone, and oily globules rose to the surface when the osazone was formed. It melted at 160°. The *p*–bromphenylhydrazine test to distinguish between arabinose and xylose was not made.

A Resume of Some Experiments on the Stems.

For the purpose of studying the properties and composition of the mucilage in prickly pear, stems of this plant, collected May 23, 1910, were sliced longitudinally, dried in the open air, and ground fine enough to enable the particles to pass a one m.m. sieve. This material was used in most of the work reported in the following pages. On account of the viscous character of the material when mixed with water, it was found to be an extremely difficult matter to separate its mucilage from the fiber and cellular debris. Some of the experiments intended to effect this separation are recorded below, together with the results of some experiments made on the several substances which were extracted by different pro-

cesses. Later experiments showed that the mucilage could be separated more readily by grinding the green stems, and first forcing the juice through cheese cloth, diluting with a volume or two of water, then filtering through muslin and finally through silk.

Treatment With Cold Water.

Ten Per Cent Extract. Ten grams of the dried sample were treated with 100 cc. of water. The material swelled up slowly, but did not form a homogeneous mass. The liquid filtered very slowly, the filtrate was not slimy, a fact which indicated that the mucilage was not in true solution. The filtrate is precipitated by alcohol, but the precipitate is curdy and not "ropy" like the true unfiltered mucilage. The filtrate reduces Fehling solution.

Ten Per Cent Extract (with Sand) Five grams of sample, 10 grams of sand, and 50 cc. of water. The object of adding sand was to determine whether it would aid centrifugal action in separating mucilage from fiber, which could not be accomplished by filtration. An extract of this strength was found to be entirely too viscous for efficient separation by this process.

Five Per Cent Extract Ten grams of sample in 200 cc. of water. After warming and shaking to obtain a homogenous thick mass the mucilage could not be separated in a clear condition in a centrifuge. When filtered under pressure through muslin and diluted with an equal volume of glacial acetic acid, making a 2½ per cent extract of the dried plant, it was sufficiently clear for a polariscope reading but it gave no rotation. The soluble solids in this solution were found to amount to 43 per cent of the dried plant. This does not include all mucilage, since that which failed to pass through muslin was thicker than the filtrate. The results for total solids are accordingly too low. Ten per cent of the dry plant was precipitated from this extract by three volumes of alcohol. The material thus obtained yielded 12.12 per cent of ash. When precipitated with three volumes of alcohol containing 10 per cent of hydrochloric acid, only 6.8 per cent of the dry matter of the plant was precipitated as mucilage. This precipitate yielded 5 to 9 per cent of ash, showing that inorganic matter was not altogether re-

moved by the acid. That part of the inorganic matter which was not removed by the acid may be a part of the mucilage molecule.

Four Per Cent Extract. Four grams of sample in 100 cc. of water. In three to four hours this mixture acquired the consistency of egg white. It was too mucilaginous to admit of separation from fiber in the centrifuge.

One Per Cent Extract. In three to four hours this formed a thick mucilage that separated from the fiber in the centrifuge, but the mucilage was opalescent and slightly turbid . Attempts to further clarify the mucilage in the centrifuge by means of sand, powdered glass, and infusorial earth were only partially successful. After the solution had been subjected to centrifugal action for several hours, it became fairly clear, and when placed in a 200 m.m. tube and allowed to settle over night had no effect on polarized light. A one per cent extract was found to clarify best by filtration through cheese cloth, first through one fold only for the removal of larger particles, then through two or more folds, and finally through silk. This solution was mucilaginous and showed no effect on polarized light. On prolonged boiling the mucilage disappeared and the solution then reduced Fehling solution, showing that hydrolysis had occurred. Apparently complete hydrolysis resulted when the one per cent extract of the mucilage was heated at 100° for ten minutes with one per cent hydrochloric acid. Attempts to clarify the mucilage and to separate it from the fibrous material by means of charcoal and aluminum cream did not prove successful. In dilute solutions the mucilage was carried down together with the other materials. In stronger solutions the mass was too viscous for these reagents to force the particles out very effectively, even in the centrifuge.

Two Per Cent Extract. Ten grams of sample added to 500 cc. of water. After standing over night the mucilage was not in homogeneous solution, but it had swollen to about one-third the volume of the water. The supernatant liquid was not slimy. To obtain the mucilage in a homogeneous condition in the water it was necessary to heat on a steam bath for a few minutes, and shake

thoroughly. In this condition the mucilage was somewhat thinner than egg white, but apparently about as viscous as the solution could be and pass, under pressure, through muslin. The liquid thus obtained was somewhat opalescent, but repeated filtration through muslin resulted in the preparation of a fairly clear solution. The solids in this solution amounted to 54.9 per cent of the total dry matter of the plant.

Treatment With Hot Water.

Five Per Cent Extract. Five grams of sample in 100 cc. of water placed in a steam sterilizer were apparently hydrolyzed in one hour, since the resulting solution had completely lost its mucilaginous character.

Four Per Cent Extract Four grams of sample in 100 cc of water. Swells to a homogeneous mucilaginous mass when heated for a few minutes on a water bath. This could not be filtered. In forcing some of it through muslin it was noticed that the portion that passed was much thinner than that which remained on the muslin.

One Per Cent Extract. One gram of sample in 100 cc. of water. When this mixture was placed in the sterilizer for one hour the mucilage was destroyed, and the insoluble material completely separated. After this treatment the liquid could be readily filtered to a clear solution that had no effect on polarized light, and heavily reduced Fehling solution.

Dialysis of the Mucilage.

An attempt was made to remove the mucilage from fibrous and cellular material by diffusion of the two per cent extract through collodion bags and parchment paper. Sugars, mineral matter, and other soluble materials were found in the diffusate, which reduced Fehling solution and yielded a precipitate with lead acetate. Diffusion of these products through the semi-permeable bag was greatly retarded by the mucilage, which was found to be indiffusible and formed a smeary lining on the walls of the membrane.

Examination of the Material Under the Microscope.

Thin cross sections from the plant showed masses of very large thin-walled cells that often contained deposits of a granular material about the size of bacteria, that were stained blue by Jenner's* process and yellow with iodine. An occasional starch granule was present that stained blue with iodine. The dried powder was also composed of large transparent cells that began to swell when water was added, and finally ruptured, allowing the solution to flow from them as a clear liquid.

Treatment With Alcohol-Ether-Water Mixtures.

For the purpose of testing the efficiency of mixtures of alcohol, ether and water in removing coloring matter and other soluble material that would prevent the proper clarification of the mucilage when dissolved in water, combinations were tried in the proportions indicated in the table below. In all cases two grams of the powdered material were treated and equal volumes of alcohol and ether were used.

* Jenner: Lancet, i, 1889.

Table I.

Proportion of Water to Alcohol–ether.	Effect on Solution	Effect on Residue
One to Nine	Clear, bright green color	No swelling, bleached
One to Eight	Clear, brownish color	Slight swelling
One to Seven	Clear, brownish color	Increased swelling
One to Six	Not clear, alcohol too weak to dissolve all ether.	Increased swelling
One to Five	Not clear, mucilaginous, ether and solids at surface	Badly swollen
One to Four	Turbid mucilaginous, ether and solids at surface	Suspended in liquid

From the general appearance of the resultant solutions and residues it was evident that a more satisfactory extraction of the plant could be made by previous treatment with ether in a Soxhlet extractor, followed by digestion of this residue with alcohol.

The amount of solids dissolved, and the apparent effect of different strengths of alcohol on the dried plant, are noted in table II.

Table II.

Per cent Alcohol	Character of Solution	Effect on Residue	Per cent Solids Dissolved
90	Clear, filters readily	Very slight	1.00
80	Clear, filters readily	Slight swelling	1.95
70	Clear, filters well	Slight swelling	2.30
60	Little cloudy, filters slowly	Increased swelling	3.11
50	Little cloudy, filters with difficulty	Increased swelling	3.33
40	Turbid, filters with great difficulty	Increased swelling	2.86
30	Turbid, filters with great difficulty	Mucilaginous	2.78
20	Turbid, will not filter	Mucilaginous	3.52

From these results it is seen that alcohol at a concentration of 60 to 70 per cent is the most efficient strength for removing the solids, without affecting the condition of the mucilage.

Treatment With Ether and Alcohol.

In all of the following experiments the dried material was first extracted with ether for 24 hours in a Soxhlet extractor. This was followed by treatment with alcohol varying from 50 to 95 per cent until the residue was colorless.

Ether Extract. Fifty grams of the substance treated with ether in a Soxhlet extractor for 24 hours yielding 2.7 per cent of material consisting of chlorophyll, fats, etc.

Alcohol Extract. The residue after the foregoing treatment was washed with about one liter of 60 per cent alcohol to remove sugars and other soluble substances as much as possible. The alcoholic solution thus obtained was evaporated to dryness before an electric fan, and the residue treated with 95 per cent alcohol, which dissolved a small amount of the material. The extract was evaporated at 40°, redissolved in 95 per cent alcohol and the solution treated with an excess of ether. The resultant precipitate was dissolved in methyl alcohol and placed in a shallow dish for the spontaneous evaporation of the solvent and the crystallization of any dissolved substance. Crystals formed in the syrup after about ten days. When 0.6677 gram of these crystals was dissolved in 20 cc. of water, a small residue was left. This solution, after filtration, gave a specific rotation of $[a]_D = -6.6°$. It was evaporated before an electric fan, and again precipitated and washed with ether. When 0.4925 gram of the syrup, purified in this manner, was dissolved in 15 cc. of water, it gave a specific rotation of $[a]_D = -7.1°$. The osazone of this syrup consisted of yellow crystalline needles, with the melting point of glucosazone.

Examination of the 60 Per Cent Alcohol Extract. That portion of the 60 per cent alcohol extract that failed to dissolve when treated with 95 per cent alcohol was dissolved in water, reprecipitated with strong alcohol, and the process repeated several times till a white powdery mass weighing 7.5 grams was obtained from 50 grams of the dried plant. This material yielded 30 per cent of ash, which was composed largely of sulphates and magnesium. The ash, when moistened, was acid to litmus and neutral to

lacmoid. The precipitate possessed slight reducing power before, and heavy reducing power after acid hydrolysis. With hydrochloric acid it gave a strong furfural odor. Analysis for pentoses showed that it contained 2.18 per cent. It reacted strongly with the Millon reagent, but contained only 0.7 per cent of nitrogen. A 0.5 per cent solution showed no rotation of polarized light. When the solution was hydrolyzed with dilute hydrochloric acid it darkened and a black flocculent precipitate was formed. The resulting solution gave a rotation of $[a]_D = +0.4°$. These reactions show the presence of large amounts of soluble salts, and a readily hydrolyzable polysaccharide that is not mucilaginous in character.

Residue from 60 per cent alcohol extract. An effort was made to remove all the material that caused opalescence, by repeatedly washing the residue with 60 per cent alcohol. When these washings were filtered through silk to remove cells and cell fragments, they were still opalescent. When it was allowed to stand over night, white particles separated out that had the appearance of granules under the microscope. Small amounts of these gave red coloration with aniline acetate, which led us to believe that they might be granules of a pentosan polysaccharide corresponding to starch. A further examination of the properties of these bodies rendered it more likely that they were particles of mucilage, caused to assume this peculiar solid spherical condition by the action of the dilute alcohol; which, like ether, forms an emulsion with the mucilage and causes the latter to separate into tiny microscopic globules. The residue from this treatment weighed 32 grams, showing that ether and alcohol had dissolved about 36 per cent of the dried plant.

Two grams of this residue dissolved in 100 cc of water and filtered first through muslin, and then through silk, and finally through a Buchner funnel with filter paper, was quite clear. The filtrate contained 0.65 per cent of solids, and had no effect on polarized light.

The Fruits.

The tuna being a perishable fruit, it became necessary to preserve the available specimens in such a manner as to prevent change in the character of the sugars originally present. For this

purpose the ripe fruits of *Opuntia dulcis* were preserved in two different forms. In one sample the juice was forced from the ripe fruits by means of a press. The juice was then evaporated to about one-sixth of its original volume in a vacuum at a temperature not exceeding 65°. The syrup obtained in this manner could be preserved indefinitely, as the proportion of sugar then present was too great to permit of fermentation.

The second sample was prepared by slicing the fruits and allowing them to dry in the open air, which was readily and speedily accomplished under the hot New Mexico sun without any apparent change in the sugars.

A third sample that was also used in a study of these sugars was a sample of "tuna miel," or prickly pear honey, a commercial article of diet among the Mexicans, prepared by evaporating the juice of the fruit to a thick syrup. This sample was used because it was found to contain large crystals of some sugar that had evidently been formed in the sample of "miel" after standing several months. These crystals were first separated as freely as possible from the mother liquor mechanically, and two and one-half grams dissolved in 25 cc of water. In this condition the solution was too dark for reading in the saccharimeter, but on filtering through charcoal, it showed a rotation of $[a]_D = +27°$. After standing three hours this was reduced to $[a]_D = +24°$, but failed to show further diminution on standing for longer periods. Calculating the specific rotation from the above figures we have

$$S\frac{100a}{l.c.} = \frac{100 \times 24 \times .3468}{2 \times 1} = 41.62$$

The specific rotation of *d*-glucose is $[a]_D = +52.7°$. The solution was evaporated in a vacuum desiccator over sulphuric acid, when small white crystals were obtained, whose melting point, while not definite, was between 77° and 100°. The osazone melted at 208°. Since glucose melts between 85° and 90°, and its osazone at 204° to 205°, the crystals were asumed to be those of impure glucose.

The syrup obtained by evaporating the juice of the fruit in the manner described above was treated with the object of sep-

arating its sugars in a crystalline condition. It was soon found to be an extremely difficult matter to crystallize the sugars satisfactorily from their solution with inorganic salts, organic acids, mucilage, etc. Two hundred grams of the syrup mixed with 200 cc. of 80 per cent alcohol gave a very dark brown solution that could not be completely clarified by repeated digestion with pure animal charcoal. Upon adding about three volumes of 98 per cent alcohol to the partially clarified solution a syrup was precipitated, but this syrup as well as the one obtained by evaportaing the unprecipitated portion in vacuum failed to crystallize after several weeks standing. Solutions of the first mentioned syrup in 95 per cent alcohol also failed to crystallize. A portion of the syrup treated with strong methyl alcohol caused the separation of a white flocculent precipitate, and the filtrate from this product yielded crystals in three weeks, when kept over sulphuric acid in a vacuum desiccator. These crystals were washed on a porcelain tile with a small amount of 98 per cent alcohol. The residue was then dissolved in 90 per cent alcohol and the solution evaporated in a vacuum desiccator. Crystals were again formed; they had a specific rotation of $[a]_D = +25.02°$. That these figures are lower than those for the specific rotation of glucose is probably due to the presence of small amounts of fructose which were not removed. The melting point of the osazones, from these crystals was found to be 210°, that for glucosazone being 204–5°.

Sugars Precipitated by Basic Acetate of Lead.

In an effort to remove the mucilage, organic acids, etc., by means of basic acetate of lead, it was noticed that if the precipitate obtained was washed with hot water until the washings failed to give any reaction for carbohydrates, and was then decomposed by means of hydrogen sulphide, the filtrate from the lead sulphide precipitate gave the Molisch test, and effected a heavy reduction of Fehling solution. This was found to be the case after several repetitions of the process of precipitation with basic lead followed by thorough washing, and decomposition of the resulting precipitate with hydrogen sulphide. These reactions indicated that an un-

identified sugar might be precipitated by the basic lead acetate. The reducing action of the solution obtained in this manner, and the fact that the solution was strongly acid, suggested the possibility of an aldehyde or ketone acid, like glyoxylic acid, etc, but when the solution was exaporated in a vacuum to a syrup it yielded crystals after several days standing, which gave qualitative tests for malic acid. The osazone of the syrup had properties of glucosazone. The sugar present in the lead precipitate was doubtless a mixture of glucose and fructose held so firmly in the body of the precipitate that they were not removed, even after several precipitations followed by thorough washing, as described.

It is well known that unusual amounts of reducing sugars occur in the precipitate with basic lead acetate from impure solutions of these sugars. The following experiments made with diluted solutions of two samples of tuna syrup show the extent to which reducing sugars are removed by both neutral and basic lead acetate solutions.

	Per cent of Reducing Sugar.	
	First Solution	Second Solution.
Solution unprecipitated	10.40	5.17
Solution after removal of precipitate produced by neutral lead acetate	8.33	4.70
Solution after removal of precipitate produced by basic lead acetate	8.09	4.11

A ten per cent solution of this sugar had little effect on polarized light, the rotation being only $+1^{\circ}$. This slight dextro rotation may have been due to the presence of glucose and fructose in the proportion to nearly compensate the rotation of each other.

Separation of the Sugars by Dialysis.

An effort was made to remove the sugars in the syrup from the associated mucilage and other non-diffusible material by dialysis through parchment. For this purpose 500 grams of the syrup were dissolved in about 500 cc of water and placed in parchment bags, and these in turn were placed in tall cylinders, which were then filled with distilled water to the level of the sugar solution in the bags. Diffusion proceeded satisfactorily at first, but in a short time the movement of sugars through the paper was very slow, because of the imprevious deposit of mucilage which soon formed on the inner surface of the bag. This slimy deposit sufficiently prevented the passage of the sugars to render this method of separation impractical. Dialysis through collodion bags proved equally unsatisfactory for the same reason.

Purification of the Sugars by Precipitation with Subacetate of Lead

Neither collodion nor parchment paper bags were very efficient in retaining all the impurities in the solution, as was seen by the precipitation from the diffusate of considerable material with both alcohol and basic acetate of lead. The carbohydrates apparently occur in the fruits in various molecular sizes, from simple monoses to the higher polysaccharides like mucilage and starch. The ripe fruits contain little carbohydrate other than sugars, but the organic acids, soluble gums, and inorganic salts which are present diffuse through parchment and are precipitated from the diffusate by basic lead acetate. The use of this reagent for the separation of impurities from the syrup seemed undesirable, because it removed some sugars, together with the impurities, and failed to remove certain substances that prevented the proper crystallization of the sugars. Then too, the subsequent removal of excess of lead with sulphureted hydrogen, or sulphuric acid, followed by exaporation of the solution for crystallization, is likely to result in transformations of the sugars. However, basic lead acetate seemed to be the most efficient substance for the purpose that could be

found. Subsequent decolorization with pure animal charcoal did not seem to aid much in the purification of the sugar.

In attempting the purification of the syrup by means of basic lead acetate, about 100 grams of the syrup were diluted with four to five volumes of water and the reagent added in excess. After filtering, sulphureted hydrogen was passed into the warm filtrate until all the lead was completely precipitated. The liquid was then filtered, boiled to remove excess of the gas, purified with animal charcoal, and evaporated in a vacuum to an amber colored syrup, This failed to crystalize after standing for several days. It was then digested with absolute alcohol several times on a water bath. Some sugars were dissolved from the syrup by the absolute alcohol. The solution darkened very speedily during the attempt to remove the alcohol in a vacuum at a low temperature. Crystals separated from the solution that failed to redissolve in 95 per cent alcohol. These crystals reduced Fehling solution, and formed an osazone with phenylhydrazine that melted at 200°. They gave the aniline acetate reaction, and, in a rather dilute solution, had apparently no effect on polarized light.

After evaporating the absolute alcohol solution to a syrup, some crystals formed on standing for several days. A 10 per cent solution of the mixed syrup and crystals gave a specific rotation of $[a]_D = -6.94°$. The solution gave reactions for fructose with resorcin and methylphenylhydrazine.

The residue from the treatment of the original syrup with absolute alcohol was treated on a steam bath with 95 per cent alcohol. The solution darkened promptly and had to be clarified with animal charcoal. It was evaporated in a vacuum desiccator over sulphuric acid. No crystals were formed in the resulting syrup. Three grams in 20 cc. of water gave a specific rotation of $[a]_D = -5.6°$.

The residue of syrup which did not dissolve in absolute alcohol, or in 95 per cent alcohol, was dissolved in water and the solution clarified with animal charcoal. It gave a specific rotation of $[a]_D = -10.4°$. It also gave the aniline acetate reaction for pentose.

Composition of the Fruits

The fruit under examination was collected August 21, 1910. Below is given the results of an analysis of a fresh sample:

Average weight of fruit in grams	24.3
Per cent of seed and mark after pressing	15.00
Per cent of juice	85.00
Specific gravity of juice	1.063
Total solids in juice (per cent)	14.06
Reducing sugars as dextrose (per cent)	11.20
Sucrose by reduction (per cent)	0.15
Polarization before inversion	+1.90
Polarization after invasion	+1.50
Acids as acetic acid (per cent)	0.045
Ash (per cent)	0.83
Alcohol precipitate (per cent)	0.25

Forty grams of the dried fruit were extracted with water. The syrup from the soluble portion weighed 24.5 grams, or 61.25 per cent of the dried fruit.

Estimation of Pentoses*

A quantitative estimation of the pentoses was made in both the soluble and insoluble portions. The former was found to contain 1.57 per cent, and the latter, freed from seed, 9.55 per cent of pentosans.

Analysis of a sample of the original syrup showed that it contained 2.06 per cent of pentoses. It cannot be stated definitely whether this sugar was present as such, or as a soluble polysaccharide, but the fact that the filtrate from the basic lead acetate precipitate gave reactions with aniline acetate, would seem to indicate the presence of at least some pentose in this fruit.

Examination for Fructose.

The soluble portion of the dried fruit gave both the resorcin and methylphenylhydrazine reactions for fructose. In the latter case the resultant osazone melted at 158° to 160° and had other properties similar to those of the methylphenylhydrazone of fructose

*A. O. A. C. Methods.

All reactions indicated its presence in considerable quantity but the amount was not determined.

Examination for Galactose.

Treatment of 10 grams of both fruit and syrup with nitric acid according to the method of the Association of Official Agricultural Chemists produced only traces of mucic acid. After the syrup had been previously purified with basic lead acetate, not even a trace of mucic acid was formed in 10 grams of the syrup. The dried mucilage of the stems and green fruits has about 15 per cent of galactans, that are evidently not hydrolyzed to galactoses in the process of the ripening of the fruits.

Examination for Glucose.

Saccharic Acid Test. Ten grams of the water soluble portion of the fruits when oxidized with nitric acid according to the method described in Allen's Commercial Organic Analysis, Vol.1, page 271, formed the characteristic potassium acid salt of saccharic acid. Another portion of five grams of the syrup was treated according to Tollens' method given in Abderhalden's Handbuch der Biochemichen Arbeitsmethoden, Vol. II, p. 106. in this case the five grams of syrup from the fruits that had been purified by precipitation with lead acetate and clarified with charcoal gave three grams of the mono potassium saccharate. If, as Tollens states, pure glucose yields 30 to 40 per cent of this salt, the foregoing result indicates that the purified syrup contains 5 to 10 per cent of glucose. The mono-potassium salt precipitated as silver saccharate and ignited as such, was found to contain 52.05 per cent of silver, the theoretical amount being 50.94 per cent.

Diphenylhydrazine Test. The syrup yielded characteristic diphenylosazones of glucose that melted at 161° to 162°.

The Coloring Matter of the Tuna.

The abundance of rich magenta pigment in the fruits of *Opuntia dulcis* suggested its possible technical value as a dye stuff and for coloring foods.

A number of experiments with silk, wool and cotton, both mor-

danted and untreated, showed this coloring matter was not sufficiently fast to be utilizd for dyeing fabrics. Experiments also proved that this pigment is not satisfactory for coloring foods and drugs when it is necessary to keep the color in solution, especially in the presence of oxidizing agents or light. For coloring ice cream, candies, fruit preserves, and various iced drinks and beverages, the tuna color has been found to be an exceedingly rich and attractive pigment. The cheap and abundant supply of the fruits, and the easy and inexpensive manner of separating the coloring matter and concentrating it to a beautiful and perfectly harmless paste for use in coloring certain food stuffs, would seem to justify its introduction into the trade. Red vegetable colors that are now used for this purpose are quite scarce and are all imported. The only desirable one now on the market retails, in the solid condition, at $6.00 per pound. A sample of this pigment was submitted to us by Fritzsche Brothers, New York City. Its tinctorial value and permanency exceed that of the tuna color in any form that we have been able to obtain it, but the brilliancy of the latter is superior to that of the former. The yield of eight to ten tons of tunas per acre, and the abundance of pigment would make its production quite profitable, if it could be marketed at fifty cents per pound and even less. If the fruits, which average about eight per cent of a fermentable sugar, should ever be utilized for the production of alcohol, the pigment could be obtained from the "spent wash" in the beer stills.

Natives of the Southwest and Mexico make rich colored preserves from these fruits. The evaporated juice contains from 30 to 60 per cent of glucose, but seems to be free from pectin substances, since it will not jell unless mixed with an abundant supply of other fruit juices that jell readily.

The rich coloring matter, with the high sugar content, of this fruit would render it valuable for the production of a cheap preserve if the source of supply was nearer the large markets.

Some Properties of the Coloring Matter.

The coloring matter of the tuna is somewhat different in its properties from that of most other vegetable colors. It is insoluble

in all the immiscible solvents, in acetone, aldehyde, ethyl acetate, and alcohol, except methyl alcohol, in which it is slightly soluble. Indeed these reagents, which are general solvents for most colors, will precipitate this pigment from its solution in the juice.

A quantity of the dried fruits was chopped into small pieces and extracted with ether, carbon bisulphide, carbon tetrachloride, petroleum ether, acetone, absolute alcohol, and 95 per cent alcohol. None of the red colored pigment was dissolved by any of these reagents, except 95 per cent alcohol, which dissolved a light red pigment, but none of the magenta color. When treated with pure methyl alcohol the red pigment was partially dissolved. Upon evaporating the solution to dryness before an electric fan, and removing some chlorophyll and other pigments by means of ether, the red coloring matter was obtained apparently in a fairly pure condition. The residue was syrupy, and reduced Fehling solution, indicating a possible mixture of some fruit sugar with the color.

The purified coloring matter, in dilute solution, is bright red with acids, changing to violet when neutral, and yellow when alkaline. The end reactions are quite sharp, but not so decided as for certain other indicators.

The fruit evidently contains several colors. Ether dissolves some chlorophyll, acetone dissolves a yellow pigment, and 95 per cent alcohol dissolves bright red material, leaving a darker magenta product undissolved. Basic acetate of lead precipitates all the pigments; netural lead acetate will only precipitate the magenta product. Silver nitrate precipitates the latter material from strong solutions, but the precipitate formed is quite soluble in water.

METHOD OF SEPARATING THE PIGMENTS

Many attempts to separate and concentrate the coloring matter of the tuna have been tried. The most efficient method seems to be to remove the mucilaginous material from the juice by the addition of one to two volumes of alcohol. In the filtrate from the above treatment two volumes of acetone precipitate the magenta pigment is as a syrupy mass. When this is dried and freed from acetone, the pigment is in quite a concentrated condition. In this state the color

is permanent and is probably the form best suited for the market. To further purify this material, a water solution can be precipitated with lead acetate. This precipitate may be washed, dissolved, and reprecipitated several times. The pigment is liberated from its compound with the lead by strong acids or potassium acid sulphate, but not by carbonic acid or sulphureted hydrogen. The color thus liberated will not be again precipitated by acetone. If evaporated to dryness in the presence of the acid, the color is destroyed and the resulting solution reduces Fehling solution. The sugar forms an osazone that melts at 205° and has other properties of glucosazone.

The lead salt of the coloring matter contains 61.42 per cent of lead.

Conclusions.

The difficulties encountered in the practical laboratory separation of the sugars from the mineral matter, mucilages, gums and dextrinoid substances have been numerous, and the operations time-consuming. Many attempts to obtain the sugars free and in crystalline form have usually resulted unsuccessfully; so that it became necessary to make the individual tests not on the sugar crystals, but on the syrups previously purified as much as possible by different methods.

The juice of the ripe fruit contains 1.57 per cent of pentosans and only traces of galactan. When previously precipitated with lead acetate, the juice gave the aniline acetate reaction for pentose, but none for galactose. The presence of fructose and glucose in considerable amounts was quite definitely established by several reactions characteristic of these sugars.

The dried mucilage of the prickly pear, when separated by precipitation with alcohol from a two per cent solution, contained 15 per cent of galactan, 31 per cent of pentosan and 12 per cent of ash.

The mucilage could not be separated completely from cell fragments, starch, crystals of calcium oxalate and other solid particles that caused opalescence and turbidity. A dilute solution with

1.5 per cent of total soluble solid matter, rendered fairly clear by repeated filtration through silk, had no effect on polarized light. This was true of all the solutions of mucilage obtained in this work, both before and after subjecting them to acid hydrolysis. Harley* reports having found a specific rotation of $+38^{\circ}$ for Opuntia mucilage, but places little confidence in his own results, since the reading was made on a very dilute opalescent solution and calculated from an observed rotation of +6 minutes.

Hydrolysis of the mucilage by digestion for several hours with 1.25 per cent sulphuric acid solution produced a sugar that had properties similar to arabinose. When its osazone was formed, oily globules rose to the surface. The precipitate was darker than glucosazone, readily soluble in hot water and melted at near 160°.

A 95 per cent alcoholic extract of the dried stems, previously treated with ether, contained a sugar with specific rotations made on three separate solutions of -6.6°, -8.25°, and -7.1°. The osazone produced from this sugar had properties similar to those of glucosazone. These results indicate the presence of mixtures of glucose and fructose in this extract.

A 60 per cent alcoholic extract of the dried stems contained a substance apparently intermediate in character between mucilage and sugars. It did not reduce Fehling solution before hydrolysis, but was very readily hydrolyzed by dilute acids. Alcohol stronger than 60 per cent reprecipitated this material as a flocculent mass, quite different in appearance and properties from the precipitate of the mucilage with alcohol. The precipitate was readily soluble in water, but its solution was not mucilaginous. When hydrolyzed it gave a plus rotation to polarized light.

The coloring matter can be concentrated and made into a marketable product, of value for coloring certain foods, by first removing mucilages and gums with alcohol, and precipitating the pigment from the filtrate with acetone.

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*Harley: Journal de Pharmacie III, 6–193.

The pigment is evidently a glucoside. When separated from the juice with alcohol and acetone, and then precipitated with lead acetate, the coloring matter liberated by sulphuric acid gave a sugar on hydrolysis, with properties similar to those of glucose.

The lead salt produced by precipitating the purified pigment with lead acetate contains 61.42 per cent lead.

Biographical Note.

Raleigh Frederick Hare was born in Opelika, Alabama, on June 6, 1870. He received his college preparatory training in the public schools of Alabama. In the fall of 1887 he entered the Alabama Polytechnic Institute, from which he graduated in 1892, with the degree of Bachelor of Science. He held a scholarship in chemistry at the above named Institute in 1892—3, and at the end of this collegiate year received the degree of Master of Science. He was appointed assistant in chemistry at the New Mexico Agricultural College in 1893, and held this position until 1903, when he was appointed professor of chemistry and chemist to the Experiment Station at this institution, which position he now holds.

During the academic year of 1910–1911 he pursued graduate work in biological chemistry and chemistry under the Faculty of Pure Science at Columbia University.