

14759
87

A CHEMICAL STUDY OF THE FRUIT OF CRATAEGUS.

A THESIS SUBMITTED TO THE FACULTY OF
THE GRADUATE SCHOOL OF THE UNIVERSITY
OF MINNESOTA, BY EARL PETTIGEON, IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE.

May, 1912.

MOM
8 P4535

1

A Chemical Study of the Fruit of Crataegus.

The genus *Crataegus*, commonly known as the Hawthorn, is a branch of the Rose Family. Its members are shrubs or small trees, with scaly bark and rigid terete branches bearing spines. They are of dense and low growth. The leaves are alternate, deciduous, stipulate, serrate, often lobed or pinnatifid.

The wood has few useful properties. It is heavy and solid, a fact which gave the genus its name. It is principally used for levers, handles for tools, etc.

The flowers are regular and perfect and resemble closely those of the fruit bearing members of the Rose Family, particularly those of the plum and cherry. The calyx is five-lobed, there are five, usually white petals, from ten to twenty stamens and a single ovary with from one to five cells, each cell containing two ovules. The flowers are pedicellate and in corymbs.

The fruit is a drupelike pome, ovate or globose,

AUG 6 1962 6.70

115767

red yellow or black usually somewhat open or concave at the summit. The sarcocarp is dry and mealy.

There are about seventy species, many of which are wild in America. They are very much crossed so that the identification of an unknown species is somewhat difficult. They are principally grown for ornamental purposes.

The tree from which the ripe fruit was obtained was about ten feet high, and two inches in diameter at the base. There was no distinct trunk, branches beginning close to the ground, and some coming from below the surface. The bark was rough to about two feet from the ground being cracked and furrowed. The upper branches were grey, showing reddish underneath and the new branches were of a dark red color. The thorns were from one to two inches in length and reddish brown in color.

A few clusters of the dry fruit still remained on the tree but most of them had fallen off. They were very much shrivelled, showing that a considerable

portion of the ripened fruit was volatile in air at ordinary temperatures.

The fruit and leaves were identified as belonging to the species *Macracantha*, which is very similar to the species *Crataega Coccinea*, having, however, smaller flowers, with narrow glandular calyx lobes and having the flowers produced in broader, looser, corymbs. The fruit is globose or subglobose, smaller and less fleshy with larger nutlets.

The green berries were obtained from a tree growing wild. Unfortunately, it was impossible to get material for the identification of this species. The fruit as obtained was of unusually large size, greenish yellow in color and not quite ripe. The results obtained on analysis, however, would tend to show that the fruit was almost, if not quite ripe at the time the experiments were run.

The first determinations run were of a general nature. The average weight of the fruit was found to be

1.88 gms.

Wt. of 5 apples = 9.3855 gms.

" " 1 apple = 1.8771 "

Of this the meat amounted to 82.30 o/o

Wt. of meat from above sample = 7.7200 gms. = 82.30 o/o
and the seeds 17.70 o/o

Wt. of seeds from above sample = 1.6655 " = 17.70 "

These determinations were made on the whole ripe berry.

The meat and seeds were then dried to constant weight at
105°. The meat showed 72.61 o/o of volatile material and
27.39 o/o solids;

Meat as above dried to constant weight = 2.114 gms
= 27.39 o/o solids.

Loss in weight = 5.606 gms.
= 72.61 o/o volatiles.

the seeds, 71.62 o/o solids and 28.38 o/o volatile matter.

Wt. of seeds taken = 1.3574 gms.

Wt. dried to constant = .9850 solids = 71.52 o/o

Loss in weight = .3904 volatiles = 28.38 o/o

In comparing the fruit with our ordinary ones,
like the apple and pear, there is a very noticeable
difference in the relative weights of meat and seed.

However, it does not differ to anything like as great an

extent from the cherry, plum, prune, or even the peach.

Moisture determinations were made by drying the whole fruit in an oven at 105°C to constant weight. On drying for four hours at 102° the loss in weight was 63.57 o/o, but the final drying to constant weight at 105°C gave a loss in weight amounting to 64.86 o/o.

$$\text{Wt. of dish + sample} = 25.3400$$

$$\text{'' '' ''} = 9.5551$$

$$\text{'' '' sample} = 15.7849$$

$$\text{Wt. of sample + dish} = 25.3400$$

$$\text{Dried at } 102^\circ\text{C Wt.} = 15.3050$$

$$\text{Loss in wt.} \quad 10.035 = 63.57 \text{ o/o}$$

$$\text{Wt. of sample + dish} = 25.3400$$

$$\text{Dried constant at } 105^\circ\text{C Wt.} = 15.0116$$

$$\text{Loss} = 10.2384 = 64.86 \text{ o/o}$$

It is very probable that the loss in weight indicated above was not entirely due to loss of water. It seems impossible to get an accurate moisture determination on material of this kind, since it was

found that even on drying over sulphuric acid in vacuum, at room temperature, there was a loss of non-water volatiles sufficient to color the acid a deep yellow. A determination of loss in weight made by keeping a weighed sample of the fruit in vacuum over sulphuric acid, for a period of about six weeks gave a loss amounting to but 59.14 o/o. This gives 5.72 o/o difference in the two results.

The next work done was on the ash present in the fruit. Several of the whole ripe berries were ashed in a weighed porcelain crucible at as low a temperature as possible, the skin of the fruit being broken so as to allow the juices to escape and vaporize. A skeleton berry, in ash was obtained in this way. The ash was practically white and the results checked well. Total ash amounted to 1.18 o/o.

Wt. of sample + cruc.=	28.5532	33.4976
" " "	= 22.3864	27.5130
" " "	----- 6.1668	----- 5.9846

Wt. of cruc. + ash = 22.4574	27.5774
" " "	= 22.3864
" "	-----
" " "	.071 = 1.15 o/o .0644 = 1.08 o/o

The weighed ash was separated into soluble and insoluble parts and qualitative tests were run. It appeared to be a normal fruit ash. In the soluble ash, phosphates, magnesium, chlorine, sodium and potassium were found; in the insoluble, carbonates, calcium, phosphates, silica, iron and manganese.

Some of the seeds were separated from the meat, cleaned, dried, weighed and then ashed. The ash in this case amounted to 1.40 o/o which would give a value of 1.06 o/o for the ash contained in the meat.

Ether Extract.

The whole ripe fruits were dried in a vacuum dessicator for about six weeks. A sample amounting to 43.282 gms. was then weighed out and extracted in a Soxhlet with ether. The extraction was continued for about six hours at the end of which time the solvent coming over had changed in color from a light green to colorless. The extract which was light green in color was filtered and the filtrate containing the extracted material evaporated to dryness. This was done at room temperature and gave 1.48 o/o extract. Two constituents, both solid, were present in it. One constituent was yellowish green and syrupy, the second dry and powdery. The syrupy constituent was soluble in water and its solution turned blue litmus paper red. On titration with sodium hydroxide the equivalent of .2 o/o of sulphuric acid was found. The dry powdery portion after washing with water, yielded a greenish white waxy substance that amounted to .67 o/o.

Wt. of dish + ether residue = 31.5168

" " " = 30.8755

" " " ----- .6413 = 1.48 o/o

" " portion insoluble in H₂O = .3345 = .67 o/o

The residue from the ether extract was dried
returned to the Soxhlet and extracted with 95 o/o alcohol.

Alcoholic Extract.

For purposes of qualitative analysis, an alcoholic solution was prepared by mashing up two or three hundred grams of the ripe fruit and then letting it stand covered with 85 o/o alcohol until extraction was judged to be complete. The solution obtained in this way was a cherry red in color, almost black by reflected light. It did not afford a very good idea of what the alcoholic solution obtained by the extraction with the Soxhlet contained, as the extraction with 85 o/o and with 95 o/o alcohol differed to a considerable extent. One constituent was extracted by the 85 o/o alcohol which was not by the hot 95 o/o, and this accounted for the difference in color of the two solutions. A corresponding difference was to

be observed in the water solution, that obtained by extraction in the Soxhlet being darker in color and containing the constituent which the hot 95 o/o alcohol failed to remove. Aside from this constituent the solutions were similar.

The total extract obtained in this way had a volume of about four liters. This was distilled to half the volume, the distillate being colorless, but having a decided non-alcoholic odor and probably containing an unidentified aldehyde.

The alcoholic solution was tested for the presence of acids. No tartaric, oxalic, acetic, benzoic, salacylic or malic acid was found.

On letting the alcoholic solution stand, a precipitate was found to separate out. It was apparently a wax, being light brown in color. It was completely soluble in ether, and on evaporation yielded a black waxy residue. It was redissolved in hot absolute alcohol, filtered and let stand. The residue on evaporation was of the same character. A portion of the alcoholic solution was poured into water, when a light yellow

colloidal precipitate formed which it was impossible to filter. The precipitate was settled out in a flocculent condition by adding sodium chloride. It became black on standing in the air and had a waxy appearance and taste, looking considerably like beeswax. It was apparently the same as the waxy material separated in the ether extraction with the Soxhlet but was slightly contaminated by the alcohol soluble material. It began to soften at 185° and melted at 198°C.

A portion of the alcoholic extract was evaporated on the water bath, and water added to form a water solution. A small amount of a dark brown powder was precipitated and filtered off. The filtrate was evaporated down and tested for the presence of a tannin. On testing a portion of the solution with bromine, a brownish precipitate forms. Stannous chloride gives a pink gelatinous precipitate. Ferric chloride gives a greenish black color with a slight black precipitate. Lime water gives a flocculent reddish colored precipitate, much the same as that formed by stannous chloride. Gelatine solution

gives a light brown colloidal precipitate which separated out on addition of stannous chloride to the saturation point, in slimy light chocolate colored masses. Pine wood moistened with hydrochloric acid gave no reaction, indicating the absence of pyrogallol. The above reactions indicate the presence of tannin, and the absence of the pyrogallol, coupled with the fact that bromine produces a precipitate, indicates that the tannin belongs to the catechol group.

On extracting the alcoholic solution with amyl alcohol, a clear yellow extract was obtained. On evaporating this down, a brown syrupy liquid was obtained which gave the above indicated reactions.

A portion of the alcoholic solution was made slightly acid with hydrochloric and then extracted with ether. The extract obtained was a clear light yellow in color but became darker as the evaporation continued, changing first to a clear orange and then to a reddish brown. It became syrupy and almost solid.

The alcoholic solution extracted with ether without the addition of acid yielded the same results.

The residue had an intense acrid taste and gave the tannin tests as indicated above.

A test for the presence of reducing sugars showed that a considerable amount was present, or at least that substances were present which were capable of reducing Fehling's solution.

The alcohol was distilled from a portion of the alcoholic solution and some hydrochloric acid was added. This was then placed in a distilling flask with a condenser attached and distilled. The distillate gave no test for acids but would reduce ammoniacal silver nitrate almost immediately, giving a heavy black precipitate without even warning.

The undistilled residue in the flask was allowed to stand and a heavy dark colored precipitate settled out. This was washed and dried. It was fine and velvety, reddish brown in color, looking considerably like ground limonite. It was almost insoluble in water, moderately strong sulphuric and hydrochloric acids. It was not acted on by cold nitric acid but was decomposed by the hot acid,

giving a yellow solution. On treating some of the precipitate with sulphuric acid and potassium permanganate, a heavy precipitate of manganese dioxide formed. On filtering this off and evaporating down the filtrate, the same yellow solution was obtained. It was almost completely soluble in ammonia and in alcohol.

The precipitate was probably a phlobaphene, which is one of the constituents into which a tannin or glucoside may split up on hydrolysis.

The next point was to determine whether any glucose was formed by this hydrolysis. In order to ascertain this fact the alcoholic extract was precipitated with lime water, which gave a reddish brown precipitate and a clear yellow filtrate. The precipitate was dried and then subjected to hydrolysis with dilute acids, constant volume being maintained. It was then cooled and filtered. A chocolate brown precipitate, flocculent, was obtained and a clear orange red filtrate. The filtrate was extracted with ether, to remove gallic acid if present.-the ether extract was colored yellow-

and then neutralized with sodium carbonate. It was then boiled to remove ether and cooled. It was then precipitated by means of basic lead acetate to remove tannin and coloring material. This left a slightly brown filtrate.

The excess lead was neutralized by means of dilute sulphuric acid and refiltered. The filtrate was again neutralized with sodium carbonate and the solution, then tested for sugars with Fehling's solution. This solution was reduced, which would indicate the presence of glucose but the polariscope gave a zero reading.

The ether extract obtained above gave a slight amount of a thick syrupy liquid, yellow in color. It was treated with ferric chloride and gave a greenish coloration. The water solution reddened very slightly on adding potassium cyanide which is a test for gallic acid.

The filtrate from the time precipitation was extracted with ether and alcohol. The extract was filtered and evaporated. The solution obtained in this was tested with Fehling's solution and showed a very

strong reduction. On farther evaporation, a syrup, light brown in color was obtained which had a sweet fruity taste. It was composed of reducing sugar and a small amount of oil.

On adding barium hydroxide to a portion of the alcoholic solution a precipitate forms at once. Silver nitrate also forms a precipitate with it. Both are non-crystalline, the first being finely divided and then second flocculent. The barium precipitate was neutralized with sulphuric acid, and the barium sulphate filtered off. The filtrate was then extracted with ether and the ether extract evaporated. It gave a brown syrupy liquid.

A test was made for the presence of glucosides but none were found. The yellow color of the solution was almost entirely removed by means of basic lead acetate and was due to a coloring matter present. The filtrate from this, on evaporation almost to dryness became yellowish again and on evaporation to dryness separated out a small amount of oily tasteless yellow liquid.

Quantitative Work.

The fruits which had been extracted with ether were dried, returned to the Soxhlet and extracted with ninety percent alcohol until the liquid passing back into the distilling flask was colorless. The extract itself was a clear deep orange color. It was made up to 500 cc. and portions of it were used for the quantitative work.

The first determination made was that of "total solids." 50 cc. of the alcoholic solution were evaporated to constant weight, on the water bath. The determination, as in the case of the moisture determination on the whole berries, was probably a trifle high, due in this case to the impossibility of completely removing the solvent and at the same time avoiding the decomposition of at least a part of the solids. The results obtained averaged 27.70 o/o

Wt. of beaker + solids = 32.2955	43.9842
" " "	= 31.1031
" "	-----
" "	= 1.1924 = 27.55
	o/o
	1.206 = 27.86 o/o

The solid matter as weighed was a reddish black syrupy mass. It was in this condition almost completely soluble in water.

Determinations of reducing sugar present as calculated from the reduction of Fehling's solution gave 12.39 o/o. This determination while fairly constant is probably entirely meaningless, since no reducing sugar could be discovered by means of the polariscope and since many substances separated out, reduced Fehling's solution but that was the only particular in which they resembled reducing sugars.

The alcoholic solution evaporated to dryness was taken up with ethyl acetate. It gave an orange yellow solution which was filtered into a tared evaporating dish. A single portion of the ethyl acetate took out all of the soluble matter as the second portion remained entirely colorless. It amounted to 1.98 o/o of the weight of the berries. It was waxy in appearance and apparently had a trace of tannin in it.

The extracted solution was dissolved in water and allowed to stand. It was then treated with dilute

hydrochloric acid in a reflux condenser. A second extraction of the cooled solution by means of ethyl acetate yielded 1.84 o/o. It was apparently the same in composition as that obtained above. Neither of the two extracts gave a trace of sugar using the polariscope but both reacted on Fehling's solution strongly.

Wt. of beaker + Ethyl Ac.res. =	35.1957	30.9515
" " "	= 35.1100	30.8718
" " "	-----	-----
	.0857	.0797=1.84 o/o
	=1.98 o/o	

$$\text{Total extract} = 3.82 \text{ o/o}$$

A determination of sucrose, run in the ordinary way, gave no trace of sugar(Polariscope).

A second portion of the solution was evaporated down as in the determination of total solids and then extracted with absolute alcohol. A yellow extract was obtained which on evaporation amounted to 23.12 o/o. A sticky creamy white, sweet tasting residue, probably a sugar, was left. It was dissolved in water and found to reduce both Fehling's and Barfoed's solutions. A quantitative separation of this substance was not obtained in this way.

Wt. of dish + ab. alcohol ext. = 28.6620
 " " " = 27.6616
 " " extract = 1.0004 = 23.12 o/o
 " " insoluble material = 27.70 = 23.12 = 4.58 o/o

The soluble residue after evaporation was dissolved in water and filtered. A yellow oily liquid was insoluble.

It amounted to about .8 o/o. It was odorless and tasteless.

Fifty cc. of the alcoholic solution was hydrolysed with hydrochloric acid in a flask fitted with a reflux condenser. A dark brown precipitate separated out which was filtered and weighed. The total insoluble residue gave 2.43 %.

The filtrate from this precipitate was made up to 500 cc. One hundred cc. were extracted with ethyl acetate, giving 3.6 o/o, corresponding quite closely with the two ethyl acetate extracts mentioned above which totaled 3.82.

Reducing sugar was run on the hydrolysed solution after neutralization of the acid present. It gave 4.85 %o. Since 12.39 %o of material capable of reducing

Fehling's solution was found previous to hydrolysis with about five per cent hydrochloric acid, and but 4.85 o/o afterwards, sucrose could not be present in any quantity, and some other substance capable of reducing Fehling's solution and decomposed by the dilute acid, must be present.

On adding sodium hydroxide to a portion of this solution, a light yellow gelatinous precipitate forms, insoluble in alcohol and ether but soluble in water. It is also soluble in an excess of sodium hydroxide. On evaporation, after solution in water, a brown soapy looking residue is left.

Barium hydroxide was then substituted for sodium hydroxide and it precipitated similarly a yellow amorphous compound. It was slightly soluble in alcohol and readily soluble in water. Barium hydroxide precipitated about twenty percent of the total alcoholic extract, while the remaining seven and a half percent was found in the filtrate. The precipitate as formed was light yellow in color but on drying in the dessicator it turned to a deep

brown, as if undergoing decomposition. On dissolving the barium precipitate in water, precipitating the barium by means of very dilute sulphuric acid and extracting the solution with ether and evaporating to dryness, a slight crystalline residue is obtained.

The filtrate contained the yellow oily substance mentioned above. It apparently is entirely unaffected by barium hydroxide.

An attempt was made to obtain the free acid in the following manner: 25 cc. of the alcoholic extract was made up to 150 cc. with absolute alcohol. The solution was then boiled to remove carbon dioxide and precipitated with 50 cc. of barium hydroxide. The precipitate was filtered off and the filtrate was tested for complete precipitation by adding more barium hydroxide. As an immediate precipitation did not occur it was judged to be complete. The precipitate was then washed with absolute alcohol and transferred to a beaker where it was suspended in absolute alcohol. Carbon dioxide was passed into the liquid for some time to

precipitate barium carbonate and produce the free acid. While bubbling the carbon dioxide through the solution it had the odor of ripe watermelon. The barium carbonate was filtered off and the filtrate slowly evaporated. A creamy white precipitate formed and was filtered off, evaporation being continued. This was continued until the solution left carbonized on farther evaporation. The precipitate obtained was not worked on to any extent. It contained a small amount of barium carbonate as was indicated by the effervescence on adding acid but there was also a powdery residue which was insoluble in hydrochloric, and nitric acids, and also in water. This was probably the free acid.

The precipitated material amounted to about 20 o/o and the residue to about 7 o/o.

Water Extract.

A water extract for qualitative purposes was made by drying the fruit used in making the corresponding alcoholic extract, and then allowing them to stand in water. About two litres of water were added and the mixture was frequently shaken. After standing for about four weeks it was filtered through linen cloth, it being found impossible to filter through any paper. A few drops of formaldehyde were added to keep it.

In this way a thick viscid liquid, light brown in color was obtained. It was not transparent. On standing for some time it settled to a thin jelly-like mass.

On adding dilute copper sulphate to the solution a heavy green gelatinous precipitate formed. Sodium hydroxide also caused a heavy precipitate to form. Alcohol did the same but the precipitate separated was brown in color. It showed a decided tendency toward separating out into small irregular masses. It is a gum.

Several tests were made on the water solution for the presence of proteids. Millon's reagent, Mayer's reagent, the biuret test, and the xanthoproteic reaction all failed to show the presence of proteid material.

Reducing sugars were tested for and found to be present in considerable amounts. In making a test for reducing sugar it was found necessary to remove the gum present by means of alcohol and subsequent filtration since it forms a slimy green precipitate with Fehling's solution. The filtrate on evaporation reduced Fehling's solution.

The precipitated gum when put into solution and then tested with Fehling's solution gave no reduction. It was found to be soluble in acids and to be reprecipitated by alkalies. It was insoluble in ether, acetone, carbon tetrachloride and benzene. It was soluble, when moist readily, in hot water. On allowing the precipitate to dry, a brown, hard gluelike substance is left. It was precipitated by lead acetate.

A determination of sucrose gave negative results.

On distilling a small amount of the water solution to which a few drops of sulphuric acid have been added, a brown flocculent precipitate separates out, leaving a very slightly yellow thick liquid. The brown precipitate, which was an impurity, carried down on precipitating the gum with alcohol, was filtered off. On adding alcohol to the filtrate, a clean white gelatinous precipitate separates.

A portion of the water solution was treated with alcohol and the precipitated gum separated. This was then placed in a distilling flask and hydrolysed with dilute hydrochloric acid. A colorless liquid and a brown precipitate were obtained. The liquid on adding sodium hydroxide became yellow in color. On adding alcohol to this a yellow precipitate similar to that obtained by adding sodium hydroxide to the alcoholic solution, results.

Quantitative Work.

The residue left after the alcoholic extraction was dried completely, returned to the Soxhlet and extracted with water until the liquid came over clear. The extract in this case was reddish brown in color. As in the case of the alcoholic solution, it was made up to five hundred cc. A drop or two of formaldehyde was added to prevent the action of bacteria.

The determination of total solids was subject to the same difficulties as in the alcoholic determination, complete drying without charring, being very difficult. The results obtained averaged 13.00 o/o.

$$\begin{array}{rcl} \text{Wt. of beaker + solid} & = & 31.6678 \\ \text{" " "} & = & 31.0987 \\ \text{" " "} & - & - - - - \\ \text{" " "} & & 0.5691 = 13.15 \text{ o/o} \end{array}$$

No trace of sucrose could be found. Reducing sugars were determined in the ordinary way and the determination is subject to the same criticism as was applied to the alcoholic solution. In making this determination the gum was removed previous to the addition

to the Fehling's solution. Reducing sugars amounted to 2.81 o/o.

The gum was precipitated by adding about ten times the volume of 95 o/o alcohol. It was filtered on a tared filter paper and weighed after drying. It amounted to 6.60 o/o.

Wt. of paper + gum =	1.5862	1.2554
" " "	1.4433	1.1123
" "	.1429 = 6.60 o/o	.1431 = 6.61 o/o

The filtrate was evaporated to dryness and the residue amounted to 5.75 o/o. A determination of the gum, using methyl alcohol instead of ethyl gave 6.50 o/o.

The residue obtained on evaporating the filtrate to dryness was dissolved in the least possible amount of water. Absolute alcohol was then added. This separates out a grayish waxy compound, which on filtering leaves a yellow filtrate. The precipitate on redissolving in water gives an orange colored solution which becomes darker on evaporation. The soluble portion amounted to 3.38 o/o and the insoluble, by difference, to 2.37 o/o.

Both on evaporation give syrupy semi-solids, the first orange colored and the second brown in color.

The water extract, on hydrolysis with hydrochloric acid, using the same method employed in hydrolysing the alcoholic solution, yielded .92 o/o phlobaphene. It was a brownish black in color and resembled the precipitate, obtained by the hydrolysis of the alcoholic solution.

The filtrate, which was tested for the presence of the gum, unsuccessfully, yielded on extraction with ethyl acetate, a clear yellow extract which on evaporation became syrupy and reddish brown in color. It had an intense acrid taste and was probably largely tannin. It amounted to 1.15 o/o.

Reducing sugars run on the hydrolysed solution gave 4.10 o/o, so that in this case there is an increase in the amount of sugars indicated after the hydrolysis, as would be expected if the gum were hydrolysed by the acid, and glucose formed.

Sulphuric Acid Solution.

The residue from the water extraction was extracted with 2 o/o sulphuric acid. It gave a viscous liquid somewhat similar to that obtained by extraction with water. It was filtered through cloth.

On neutralizing the sulphuric acid present by means of sodium hydroxide, a heavy gelatinous precipitate separates. It proved to be the same gum that was obtained from the water extract, but in this case it was a clear white in color.

A portion of the extract was distilled and to the undistilled residue after cooling, strong alcohol was added. A clear white clotted gelatinous precipitate formed. This was filtered off, washed and suspended in alcohol. It was found to be insoluble in alcohol and ether, soluble in acids and reprecipitated by alkalies. It was soluble in hot water.

The solution was found to contain a trace of reducing sugar.

Quantitative Work.

Very little quantitative work could be done on this solution but it is probable that little in addition to the gum and the reducing sugars was extracted. The total extract could not be determined since sulphuric acid present carbonized the solid material present, long before dryness was reached.

A determination of gum present in this extract gave 2.61 o/o.

$$\begin{array}{rcl} \text{Wt. of paper + gum} & = & 1.1004 \\ \text{" " "} & = & .9876 \\ \text{" " "} & = & .1128 = 2.61 \text{ o/o} \end{array}$$

An additional 1.30 o/o was obtained by shaking out the insoluble residue with water, filtering the gum precipitated by adding a large excess of alcohol, on a tared filter, drying and weighing in the usual way.

Results on Ripe Berries.

Ether Extract.	1.48 o/o
Acid as H ₂ SO ₄ .	.2 o/o
Waxy residue	.67 o/o
Alcoholic Solution	27.70 o/o
Reducing Sugars	12.39 o/o
Methyl Acetate Ext. total	3.42 "
Oil	6.80 "
Insol. Residue on Hydrolysis	2.43 "
Reducing Sugars on Hydrolysis	4.85 "
Barium Hydroxide Precipitate	20.00 " (op)
" " not precipitated	7.70 " "
Soluble in absolute alcohol	23.12 " "
Insoluble" " "	4.58 " "
Water Solution	13.00 o/o
Reducing Sugars before Hydrolysis	
	2.81 "
" " after	4.10 "
Gum	6.60 "
Non-gum soluble in absolute alcohol	= 3.38

Water Extract cont.

Non-gum insoluble in absolute alcohol = 2.37 o/o

Precipitate on hydrolysis .92 ''

Ethyl acetate soluble after hydrolysis 1.15 ''

Sulphuric Ext.

Gum	3.91 o/o
-----	----------

Total	46.09 ''
-------	----------

49.08

Insoluble Residue as dried and weighed-----

95.17 ''

Approximate loss in weight of seeds during extraction and approximate undetermined material is 7.5 o/o to 10 o/o.

On one of the series of determinations made, the pulp left after the water extraction was removed and dried at as low a temperature as possible to a constant weight. The pulp including the seeds gave a percentage of 52.99. The determined amount of material obtained from the sulphuric acid solution being 3.91, subtracting this from 52.99 would give the percentage weight after this extraction or 49.08 o/o. Since in the original determinations the weight of seeds was 17.70 and that

of non water constituents 40.86 % the weight of the seeds would be 43.32 %. With no loss in weight of the seeds during the extraction, about 6 % of the total material present is unaccounted for in the extracts. From 7.5 % to 10 % seems probable for the undetermined material, which is probably largely cellulose and proteid material.

Green Fruit.

The green fruit were, in general, given the same treatment as were the ripe. It was necessary, however, to keep the fruit for some time in alcohol, prior to the making of the extractions. The fruit which was dried and used for the quantitative work had therefore been partly extracted with alcohol. As a result of this the ether and alcohol results, particularly the ether, may not be exact. The determinations given on the alcoholic solution are a combination of those obtained from the Soxhlet solution with those run on the alcoholic solution, in which the green fruit was kept.

The green fruits were, as a whole, larger than the ripe ones, and more solid, the average weight of the

berries being about two grams. The ash was found to be a little higher than in the ripe, amounting to about 1.34 o/o. Its composition was just the same.

Wt. of crucible + sample	= 35.2250	32.2290
" " "	= 25.2622	22.3918
" " "	= 9.9628	9.8372
Wt. of cruc. + ash	= 25.3900	22.5276
" " "	= 25.2622	22.3918
" " "	= 0.1278 = 1.29 o/o	0.1358 = 1.38 o/o
Average	= 1.34 o/o	

The determination of moisture was subject to the same error as was noted for the ripe fruit but since the fruit of the green berry was stiffer and firmer, the loss in this case was much less. The loss in weight found amounted to 58.38 o/o. The ratio of seeds to flesh and of the amount of volatile matter in flesh and seed was not run on account of the necessity of putting the fruit in alcohol to keep it in a proper condition to work on.

Ether Extract.

A sample of the fruit which had been partially extracted with alcohol was dried in a vacuum dessicator, and weighed. It was then transferred to the Soxhlet and completely extracted. It amounted to 36.9166 grms.

The ether extract obtained resembled that yielded by the ripe berries. It consisted of a white waxy constituent and a green syrupy one. It amounted to but .86 o/o.

Wt. of beaker + ether extract = 32.9183	
" " "	= 32.6014
" "	-----
" "	= .3169 = .86 o/o

Alcoholic Extract.

Extracts for qualitative and quantitative work were made up as before and in general were similar to those obtained from the ripe fruit.

The extract used for qualitative purposes was a deep reddish brown color. The solution reduced Fehling's and Barfoed's solutions readily.

Tannin was tested for as in the ripe berries and gave corresponding tests. Ferric chloride gave a greenish

black color, lime water a reddish brown precipitate, bromine water a yellowish brown precipitate, potassium bichromate, a reddish brown one and so on.

A portion of the extract was boiled in a flask fitted with a reflux condenser, 6 o/o of hydrochloric acid being added. A dark reddish brown precipitate settled out.

The filtrate obtained by filtering off this precipitate was extracted with several solvents successively. Ethyl acetate was first used. This on evaporation gave a slight yellowish residue which responded to the ferric chloride test. Ether gave a slight amount of the same residue. Benzene gave no residue. Carbon tetrachloride gave a very slight crystalline residue. The crystals were hexagonal and slightly yellow in color. They were very highly refractive. Their melting point was 118°C. The crystals amounted to about .35 o/o and such small amounts were obtainable under the working conditions that the identification could not be made. Chloroform gave no residue.

The solution was then made ammoniacal and the extractions were repeated. No residue was obtained except with ether which yielded a very small residue similar in character to that obtained from the acid solution.

Quantitative Work.

The residue from the ether extract was dried, returned to the Soxhlet and extracted with alcohol. The extract was a golden yellow color neutral in reaction toward litmus.

Total solids yielded 24.99 %. The solids as before were similar to ordinary molasses or wine solids, except that they lacked the sweet taste. They had a characteristic plant taste and were odorless.

Reducing sugars gave 9.72 %. When the green fruit was first obtained it gave a blue color with iodine but after standing in alcohol no starch test could be obtained. The solution also reduced Barfoed's solution. The polariscope readings showed no evidence whatsoever of the presence of sucrose.

One hundred cc. of the solution was evaporated down to remove alcohol and then boiled with 6 % hydrochloric

acid using a reflux condenser. A brown powdery deposit was formed. Boiling was continued for about four hours. The solution was then cooled, filtered on a tared filter, paper thoroughly washed, dried and weighed. It gave 2.29 o/o.

$$\begin{aligned} \text{Wt. paper + precipitate} &= 1.1078 \\ " " &= .6124 \\ " " &\quad \text{-----} \\ " &= .4954 = 2.29 \text{ o/o} \end{aligned}$$

The filtrate was made up to 500 cc. and reducing sugars determined on an aliquot portion. It gave 4.66 o/o, a result considerably lower than that obtained on the original solution, and again indicating the presence of other compounds capable of reducing Fehling's solution.

This experiment was repeated, boiling for about ten hours instead of four. The insoluble residue in this case was lower amounting to only 1.82 o/o.

$$\begin{aligned} \text{Wt. paper + precipitate} &= 1.4940 \\ " " &= 1.1005 \\ " " &\quad \text{-----} \\ " &= .3935 = 1.82 \text{ o/o} \end{aligned}$$

It was slightly darker than the former product but aside

, from that fact appeared to be the same. Reducing sugars determined on the filtrate as before, yielded only 3.20 o/o.

Two hundred cc. of the filtrate from the above solution was extracted with benzene, carbon tetrachloride and ethyl acetate. Carbon tetrachloride on evaporation gave a slight crystalline precipitate amounting to .35 o/o.

Wt. dish + prec.	=	24.1421
" "	=	24.1166
" "	=	.0255 = .35 o/o

Ethyl acetate gave a gummy, or thick syrupy precipitate which was not weighed.

Water Extract.

The solution obtained in the case of the green fruit was very similar to that from the ripe. It contained some reducing substance and considerable gum. An attempt was made to steam distill some of the solution in an effort to obtain the gum in a pure state, but it was unsuccessful since only a trace of material was carried over during the distillation.

Total solids as determined gave 13.59 o/o, the solids resembling closely those obtained before. The gum present amounted to only 4.17 o/o and the reducing sugars to but 3.29 o/o.

$$\text{Wt. filter paper + prec.} = 1.1170$$

$$\begin{array}{rcl} \text{"} & \text{"} & \text{"} \\ & & 1.0402 \\ & & \hline \\ & & .0768 = 4.17 \text{ o/o} \end{array}$$

One hundred cc. of the water solution was hydrolysed. After hydrolysis no gum could be precipitated on adding alcohol. A brown precipitate similar to that obtained by treating the alcoholic solution in the same way amounted to but .77 o/o.

$$\text{Wt. paper + prec.} = .7129$$

$$\begin{array}{rcl} \text{"} & \text{"} & = .7015 \\ \text{"} & \text{"} & \hline \\ \text{"} & \text{"} & = .0114 = .77 \text{ o/o} \end{array}$$

The gum probably hydrolyses to produce the brown precipitate.

Reducing sugar was also run on the hydrolysed solution. It gave 4.10 o/o.

Sulphuric Acid Extract.

The sulphuric acid solution was not tested qualitatively as it was very similar to the same extract from the ripe fruit. Quantitative determinations were run on gum and reducing sugars. The gum gave 3.89 o/o considerably more than was found in the ripe fruit.

Wt. paper + gum = 1.1765

" " = 1.0329

" " = .1436 = 3.89 o/o

Reducing sugars yielded only .68 o/o. This was not starch inverted by the sulphuric acid since no starch could be detected in the fruit when tested microscopically with iodine.

Results.

Ether Extract	0.86
---------------	------

Alcoholic Extract	24.99
-------------------	-------

Reducing Sugars	9.72 o/o
-----------------	----------

After hydrolysis, Reducing Sugar	-4.54 "
----------------------------------	---------

Ethyl Acetate Ext. on hydrolysed Prep.	1.79 o/o
--	----------

Ba(OH) ₂	was not tried
---------------------	---------------

Water Extract	13.59%
Gum	4.17 %
Reducing Sugar	3.29 "
" " after hydrolysis	4.10 "
Brown precipitate	0.77 "
Sulphuric Acid Extract	
Gum	3.29 "
Reducing Sugars	0.68 "

In a consideration of the work as it stands, it must be stated that it is introductory and only that. The separations outlined are not known to yield pure material and the percentages given are not on the pure substance. They are simply on separated material which may have two or three constituents or only one and that one in a fairly pure or a decidedly impure condition.

The work done on sugar determinations gave promise of interesting results, if continued. If, as it appears from the results obtained, the hawthorn is in reality low in reducing sugars although it shows a fairly large

amount when the determination is run in the ordinary way, then the method used is not applicable to material of this kind, without modification. If the unmodified method is inapplicable, a great many results of reducing sugar determinations made on fruits are incorrect, if they are intended to indicate reducing sugars and are misleading in any event. It seems probable that there may be a great many compounds which are non-sugars and which will reduce Fehling's solution. Tannins would probably act in this way, if not removed previous to the determination of reducing sugars.

One serious fault could be found with the method used in this piece of work, and that has to do with the amount of material worked on. 50 gram samples were subjected to extraction and of necessity some products obtained were too small to do anything at all with. If 500 gram samples could have been subjected to the same processes, more satisfactory results would have been obtained. *xx*