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THE COMPOSITION OF PECTIN: A PRELIMINARY REPORT ON THE DETERMINATION OF GALACTURONIC ACID IN PECTIN

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RECEIVED AUGUST 17, 1925 PUBLISHED JANUARY 8, 1926

Pectic substances differ greatly in their properties and behavior and there is reason to believe that this variation is, to a large extent, related to variation in composition. Since very few quantitative data as to the composition of pectin are available in the literature, the author has undertaken an investigation to determine the proximate composition of pectic substances. So far, the work has been largely confined to the development of analytical methods. In this paper is presented a preliminary report on the determination of galacturonic acid in pectin. As the work is still incomplete, the results are offered with all due reserve.

Pectin substances have long been recognized as carbohydrate derivatives possessing acid properties. As a result of Ehrlich's[1] work, the group to which pectin owes its acid properties is now definitely known to be galacturonic acid. All pectic substances, then, contain galacturonic acid as the essential constituent. The importance of being able to determine the galacturonic acid content of pectin is accordingly indicated.

There are two reactions of galacturonic acid upon which we might hope to build quantitative methods for its determination. On oxidation with nitric acid, it yields mucic acid, $COH.(CHOH)_4.COOH + O \longrightarrow COOH.(CHOH)_4.COOH$, while on distillation with hydrochloric acid, it yields furfural, $COH.(CHOH)_4.\boxed{COO}H \longrightarrow C_5H_4O_2 + CO_2 + 3H_2O$. Since galactose also gives the mucic acid, and arabinose the furfural reaction, and since both of these substances occur in pectin, it is obvious that neither the mucic acid yield nor the furfural yield of pectin is a measure of its galacturonic acid. The second reaction, however, forms a basis for its quantitative determination by measuring the carbon dioxide which is produced simultaneously with the furfural. This reaction is specific for the carboxyl groups and is apparently not given by any constituent of pectin except galacturonic acid.

For the determination of galacturonic acid in pectin substances, the author has used a modification of the Lefèvre and Tollens[2] method for glucuronic acid. The details of the apparatus and method will be given in a future paper.

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[1] Ehrlich, *Chem.-Ztg.*, **41**, 197 (1917).

[2] Abderhalden, "Handbuch der biochemischen Arbeitsmethoden," Urban and Schwarzenberg, Berlin, **1910**, Vol. 2, p. 139; *Ber.*, **25**, 2569 (1892); **40**, 4513 (1907); *Z. physiol. Chem.*, **44**, 388 (1905).

Briefly stated, the method consists in decomposing the substance by heating with 12% hydrochloric acid (d., 1.06) and collecting the evolved carbon dioxide in a weighed Geissler potash bulb. The decomposition is carried out in a 1-liter flask under a reflux condenser. The carbon dioxide is swept out of the flask, through the condenser and into the potash bulb by a current of air free from carbon dioxide. Other products of the reactions are largely returned to the flask by the reflux condenser; the small amounts that escape condensation are prevented from entering the potash bulb by passing through a train which consists of (1) a U-tube containing aniline and a few drops of concd. hydrochloric acid for the absorption of furfural, (2) a straight tube of granular zinc for the absorption of hydrochloric acid and (3) a calcium chloride tube for the absorption of moisture.[3]

The results are shown in Table I.

TABLE I

GALACTURONIC ACID CONTENT OF VARIOUS PECTIN PREPARATIONS

Preparation	Nature	Galacturonic anhydride ($C_6H_8O_6$), %
A. "Dearabanized" beet pectin, Sample 1	Ehrlich's "crude pectin" extracted with 70% alcohol	39.66, 41.60
B. "Dearabanized" beet pectin, Sample 2		44.48, 42.62
C. Impure "Cytopectic acid" from beet pulp	Prepared as by Clayson, Norris and Schryver[4]	41.10, 41.10, 44.82
D. Pectic acid from beet pulp	"Crude pectin" demethylated with NaOH, acidified and precipitated with alcohol	54.30
E. Hydrolyzed beet pectin	Autoclaved with 1% H_2SO_4 at 120°, neutralized with $BaCO_3$ and precipitated with alcohol	61.26, 59.20

These results are, in general, considerably lower than those reported by Nanji, Paton and Ling[3a] for similar materials, probably because of secondary products in the impure preparations used. It is to be noted that these authors found that the uronic content of apple pectinogen increased upon purification from 55.16% to 74.24%.

A sample of commercial pectin, when examined by this method, gave irregular results, apparently due to the production of abnormally large amounts of carbon dioxide by secondary reactions. The reason for this irregularity is to be investigated.

Since it is desirable to have an independent galacturonic acid method for checking the results, as well as one that is more convenient and more generally applicable, attempts were made to utilize the furfural reaction in such a way as to avoid the interference of arabinose. So far, the experi-

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[3] This method has been used by the author for more than a year. Nanji, Paton and Ling [(a) *J. Soc. Chem. Ind.*, **44,** 253T–258T (1925)] have recently reported the use of a similar method for the determination of acid groups in pectic substances in which the carbon dioxide is collected in standard barium hydroxide solutions and determined by titration.

[4] Clayson, Norris and Schryver, *Biochem. J.*, **15,** 643 (1921).

ments to that end have been unsuccessful, but it is believed that the results are of sufficient interest to justify presentation at this time.

According to Ehrlich,[5] even so mild an hydrolysis as boiling with water splits off the araban portion of the pectin complex in a form soluble in 70% alcohol. Since all ordinary pectin preparations are produced by a treatment at least as drastic as boiling with water, it would appear that the arabinose-containing fraction of pectin could be leached out by digesting in 70% alcohol. The furfural yield of the residue might then be a measure of its galacturonic acid content.

In order to determine whether a sharp separation of furfural-yielding substances takes place as claimed by Ehrlich, four successive digestions were made on 2 g. of beet pectin, using in each case 100 cc. of cold 70% alcohol and allowing it to stand overnight. The following values were obtained, the quantities being expressed in percentages of original pectin.

First digestion, dry matter extracted, 13.74, furfural yield of extract, 5.17
Second digestion, dry matter extracted, 8.60, furfural yield of extract, 3.08
Third digestion, dry matter extracted, 1.63, furfural yield of extract, 0.35
Fourth digestion, dry matter extracted, 1.62, furfural yield of extract, 0.35

These results show that after two leachings with 70% alcohol, the amount of soluble furfural-yielding matter becomes small and constant, and it may be inferred that the furfural-yielding substance which remains in the residue is of a type different from that which was dissolved out.

To determine whether any quantitative relation exists between the galacturonic acid content of pectin substances and the furfural yield of

TABLE II

RELATION BETWEEN GALACTURONIC ACID AND FURFURAL YIELD

	Preparation		A	B	C	D	E
1.	"Dearabanized" beet pectin, Sample 1	Max.	41.60	19.52	18.96	2.13	2.20
		Min.	39.66	19.50	18.94	2.03	2.09
2.	"Dearabanized" beet pectin, Sample 2	Max.	44.48	18.70	17.78	2.45	2.64
		Min.	42.62	18.14	16.78	2.23	2.40
3.	Impure cytopectic acid, from beet pulp	Max.	44.82	19.05	17.88	2.39	2.52
		Min.	41.10	18.72	17.75	2.15	2.30
4.	Pectic acid from beet pulp	Max.	54.30	17.76	...	3.15	..
		Min.	...	17.22	16.82	3.08	3.23
5.	Hydrolyzed beet pectin	Max.	61.26	14.25	...	5.08	..
		Min.	59.20	12.05	...	4.15	..

Column heads: A—Galacturonic anhydride ($C_6H_8O_6$) by modified Lefèvre method. B—Total furfural yield: total phloroglucide expressed as furfural. C—Actual furfural yield: alcohol-insoluble phloroglucide expressed as furfural. D—Ratio, galacturonic anhydride to total furfural, (A:B). E—Ratio, galacturonic anhydride to actual furfural, (A:B). (In percentages of pectin substance.)

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[5] In C. G. Schwalbe's "Chemische Untersuchung Pflanzlicher Rohstoffe," Verlag der Papier Zeitung, Berlin, **1920,** p. 94.

the residue after extraction with 70% alcohol, preparations which had been analyzed by the modified Lefèvre methods were extracted twice with 70% alcohol and the residues were subjected to furfural distillation by the official method. The results are expressed as "total furfural yield." Most of the phloroglucide precipitates were then extracted with alcohol to remove methyl furfural and oxymethyl-furfural derivatives. The furfural corresponding to the weight of residual phloroglucide is expressed as "actual furfural yield." The ratios between galacturonic acid and total furfural yield, and between galacturonic acid and actual furfural yield have been calculated. The results are given in Table II.

While there is a fair agreement between the ratios calculated from duplicate determinations on the same sample, there is considerable variation in the ratios for different types of pectin bodies, although they were all prepared from the same natural source. This is true whether the comparison be made upon ratios calculated from "total furfural" or from "actual furfural." These results indicate that it is improbable that a general factor or a simple method of calculation can be found for arriving at the galacturonic acid content from the furfural yield of the alcohol-insoluble portion of pectin. Further studies on the furfural yield of pectin may provide a basis for interpreting these results.

A recent paper by Nanji, Paton and Ling[3a] offers some suggestions that may help to explain these data. According to these investigators, galacturonic acid probably occurs in a hexa-ring composed of four galacturonic acid units, one anhydro-arabinose unit and one anhydro-galactose unit. It would appear scarcely conceivable that the arabinose could be removed from such a ring by the simple solvent action of 70% alcohol. On the other hand, consistent with the views of these investigators, and with the findings of this paper, it would appear that 70% alcohol might remove arabinose units that are extraneous to the ring. If the residue contained only the ring with a constant proportion of galacturonic acid and arabinose units, we should expect that it would give a constant furfural yield. Since it does not, we may conclude that either: (1) the composition of the ring is variable, or (2) the residue contains other furfural-yielding substances than those in the ring, and these being present in varying proportions cause variations in the furfural yield. The second view appears to the author to be the more probable.

In this investigation, crude preparations were purposely used because one of the objects of these experiments was to learn whether simple extraction with 70% alcohol would yield a product of constant composition from miscellaneous impure pectins. The results show that such is not the case and indicate the necessity for further investigation with material purified in other ways. Studies with carefully purified materials are now under way.

Summary

1. A modification of the Lefèvre-Tollens method for glucuronic acid has been applied to the determination of galacturonic acid in pectic substances. The method is described and results for several pectin preparations are given.
2. The same samples were extracted with 70% alcohol and the furfural yields of the residues were determined. Comparison of the results by the two methods shows that apparently no simple relation exists between galacturonic acid and furfural yield so obtained.
3. A possible interpretation of the data is discussed with reference to Nanji, Paton and Ling's hexa-ring structure for the basal unit of pectic substances.

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STUDIES ON ATMOSPHERIC OXIDATION. I. THE OXIDATION OF GLUCOSE AND RELATED SUBSTANCES IN THE PRESENCE OF SODIUM FERRO-PYROPHOSPHATE

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RECEIVED AUGUST 27, 1925 PUBLISHED JANUARY 8, 1926

It has recently been shown[1] that various monosaccharides, disaccharides and polyatomic alcohols are oxidized by atmospheric oxygen in the presence of a complex iron phosphate catalyst and disodium phosphate. These oxidation reactions have been further studied with the thought that they would serve as a "model" of carbohydrate oxidation and throw some light on the intricate chemical reactions comprising respiration. The significance of iron, as well as manganese and copper, in respiratory oxidation has long been recognized; the kinetics of the reactions involved remains to be solved. The substances oxidized (alcohols, carbohydrates, acids) are not autoxidizable. It is the iron which reacts with molecular oxygen, and in turn with the organic substance. For iron to act thus as a catalyst certain conditions pertaining to the form of the iron as well as the dissociation of the oxidizable substances must be met. The problem has been approached from two sides. The first method is a study of the nature and action of the catalyst, with which this paper deals; the second is an investigation of the action of sodium phosphates on the dissociation of various carbohydrates, the results of which will be presented later.

The Iron Catalyst

The catalyst used is sodium ferro-pyrophosphate, made by dissolving ferrous sulfate in sodium pyrophosphate. Some of the properties of this

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[1] Spoehr, THIS JOURNAL, 46, 1494 (1924).