

# Chemical Study of Beet Pectin

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Considerable attention has been given recently to the by-products of the sugar beet, aiming at its better utilization. It is recognized that the by-products are good sources of various chemical compounds with industrial possibilities. Our present investigations are concerned with one of these by-products—the pectin extractable from the pulp.

The chief use for pectin, currently, is in the food industry as a jellying agent. Many medical applications are possible (6)<sub>2</sub>; to mention a few—in treating stomach and intestinal disorder with an apple diet the effective constituent is the pectin (9) contained therein; pectin has healing properties and plays a role in the detoxication mechanism (7) by means of its chief constituent, galacturonic acid; pectin works as an active coagulent (3) (hemostatic agent).

The main sources of industrial pectin in the United States are citrus fruit waste and apple pomace. The physical properties of citrus and apple pectins, such as viscosity, jelling qualities, etc., differ greatly from the pectin of the sugar beet. The possibility of utilizing sugar beet pectin as a constituent of jellies has been generally dismissed on account of its poor jelling qualities. However, according to recent private communications, beet pectin has been used on a large scale for jelly-making in Germany during the past war years—probably one of their many "ersatz" materials. Even if pectin from the beet is inferior for making jellies and jams, it could be used for other purposes such as pharmaceutical preparations, thickening agents, source of galacturonic and tartaric acids, etc.

Pectin is present in many plants and as a hydrophylic colloid plays a role in the translocation of water. The quantity of the pectin diminishes through the ripening process, and enzymatic hydrolysis of the pectin results in softening of the fruit. However, this transformation is not observed in the sugar beet. The seasonal variations are not significant and the mature beet at harvest time has as hard a texture as the unripe.

The pectin, as present in the plant, is called protopectin and is insoluble in cold water but can be extracted with warm water, acid, basic or salt solutions. The rate of extraction is determined princi-

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pally by pH; the greater the deviation from the neutral point, the faster the extraction.

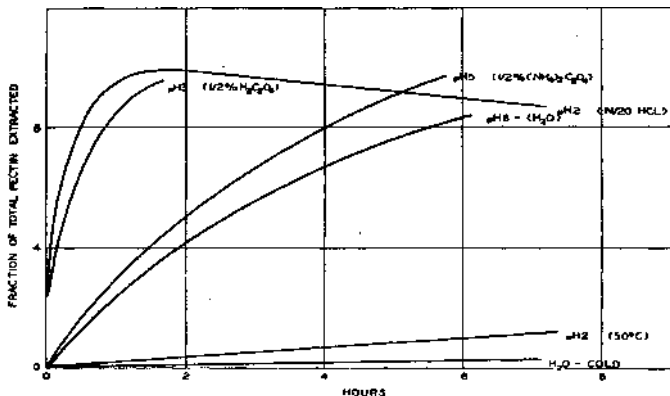
The constitution of the pectin, which is a complex polysaccharide, is not yet exactly known. According to Ehrlich (4), who did the most extensive research on beet pectin, it is an ester-acid with two of its carboxyls bound with two molecules of methyl alcohol in an ester linkage in cyclic arrangement of four galacturonic acids, called tetragalacturonic acid; arabinose and galactose are part of the pectin molecule. Other investigators came to the conclusion that the arabinose and galactose are loosely attached to the galacturonic acid unit (5, 13).

An extreme conception of the role of arabinose and galactose was expressed by Schneider (12), who reported that the presence of arabinose in the pectin molecule depends only on its solubility—if treated with 70 percent alcohol several times, more arabinose is removed each time. After five to six reprecipitations, an equilibrium is established between arabinose and pectin; then, by changing the ratio of the percentage of alcohol, the arabinose is removed completely and a purified pectin free of arabinose and galactose (galacturonic acid, 96 percent; CH<sub>3</sub>OH, 11 percent; arabinose, 0 percent; galactose, 0 percent) is obtained. According to Schneider, this highly purified pectin has good jelling properties. Schneider disagrees with Ehrlich's conception that pectin is built on a frame of four galacturonic acids and reputes his cryoscopic molecular weight determinations as being too low. Through molecular weight determinations carried out on the nitroproduct of pectin, Schneider obtained values between 20,000 and 100,000. The treatment with nitric acid might result in lower molecular weight due to oxydative degradation, but never in a higher one. Consequently, the molecular weight of the pectin before nitration should be higher. According to him pectin consists of eight galacturonic acids.

Bauer and Link (1) have been given 10 galacturonic acids as a unit, in pectin, proved by complete methylation and hydrolysis of the methyl-ester and determination of the end group.

X-ray studies (8, 2, 11) are also in contrast to the Ehrlich's work because they indicate a long chain molecule somewhat similar to cellulose. In Ehrlich's presentation every pectin is a well-defined compound, and the ratio of galactose and arabinose to the galacturonic acid must be in agreement with his formulae; if not, the pectin is degraded because of fermentation or hydrolysis. According to our present knowledge, the composition of pectin varies depending on the origin and method preparation.

We are attempting to prepare highly purified pectin from beet pulp in order to investigate structural composition and relationship.



### Experimental Parts and Description of Results

In this paper we shall outline our investigations to date on the preparation and properties of beet pectin.

The effects of time, temperature, pH, and solvents on the rate and amount of extractables of dried pulp were studied. The results are presented in figure 1 and table 1. Crude pectin is the portion of total extractable precipitated at pH 2-3 in a 66 percent volume of alcohol water medium. By the alcohol water soluble fraction is meant that portion remaining in solution after precipitating the crude pectin.

Table 1.—Extraction of pectin.

Solvent used	pH	Temp. °C	Time in hours	Ratio pulp/solvent	Soluble in alcohol-water mixture	Insoluble: crude pectin
Hydrochloric acid	5	90°	5	1:20	14.0%	84.0%
Ammonoxalate	5	90°	5	1:20	6.4%	84.0%
Hydrochloric acid	2	90°	2	1:10	14.4%	43.0%
Ferrosulfate	4	80°	2	1:10	0.0%	22.0%
Hydrochloric acid	2	80°	4	1:20	24.0%	32.0%
Water	6.5	90°	0	1:20	13.5%	27.0%

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Table 2.—Composition of pulp.

Pulp	Dry substance (percentage)	Ash (percentage)	Nitrogen (percentage)	CaO (percentage)	CaO in ash (percentage)	Galacturonic acid (percentage)	Arabinose (percentage)
Pressed	20.8	2.90	1.61	0.92	31.1	27.25	25.00
Dried	91.7	4.0	1.76	1.32	35.8	32.70	17.50

We can see from the figures that the acidity and temperature are the dominating factors in effecting a rapid extraction of the pectin from its pulp. If the extraction is continued beyond the optimum, or if more concentrated acids are used (2 1/2 percent), the yield of crude pectin is decreased, probably the result of acid degradation. The smaller amount of nonpectin extractables in the cases of water, ammonium oxalate, etc., are ascribed chiefly to the lesser solubility of the various constituents of the parent pulp.

Our standard procedure for obtaining representative amounts of pectin consists in extracting a 1/20 pulp to liquid ratio with n/20, normal HCL, at 90° C, for 1 1/2 hours with constant stirring. While still warm the pulp is pressed out, cooled to approximately 32° to 34° C, the pH raised to 4, and clarifying enzyme added for 50 minutes. These enzymes destroy starches and proteins. After 50 minutes the solution is warmed to counteract the further effect of the enzyme on the pectin.

For these extractions we used pressed pulp and dried pulp. The chemical composition of the pulp and the pectin was determined in order to ascertain the effect of drying on the composition of the pectin. The analytical data are presented in tables 2 and 3.

According to these data there is no significant difference between the two pulps—pressed and dried—so that there is no apparent adverse effect of drying; however, the preparation of pectin from the pressed pulp is an easier operation, the color of the extract is lighter, and the filtration is quicker.

Table 3.—Pectin from pressed and dried pulp.

Pulp	Ash (%)	Arabinose (%)	Galacturonic acid (%)	Methoxyl content (%)
Pressed	2.89	9.05	64.5	8.85
Dried	5.07	13.08	67.8	8.37

Table 4.—Recovery of pectin.

Treatment: Alcohol used for precipitation by volume (percentage)	Pectin recovered at pH 2-2 (percentage)	Additional pectin recovered at pH 8 (percentage)	Total recovered pectin (percentage)
50	57	24	81
65	75	10	85
70	79	7	86
80	86	6	92
76 (acetone)	87	13	100

In table 4 we present data of the recovery of pectin. The recovery of pectin is carried out by means of precipitating with ethyl alcohol. In our experiment we used various concentrations of alcohol at different pHs. The best recovery was accomplished with 80 per cent alcohol. The higher the alcohol concentration the more pectin is precipitated, but this pectin is more impure because other alcohol insoluble substances are present.

The influence of enzyme to assist in the preparation of (table 5), pectin prepared from pressed pulp and also from dried pulp was studied. The higher galacturonic acid and methoxyl content of the enzyme-treated products indicates that enzymes are a good means of improving the extract before precipitation of pectin.

The chemical constituents of the acid-treated pectins are compared with the alkaline-treated ones in table 6. As seen from the figures, the methoxyl content of the alkaline-treated pectin is much lower

Table 5.—Influence of enzyme treatment.

Condition of extraction	Ash (%)	Arabinose (%)	Galacturonic acid (%)	Methoxyl content (%)
Pectin from pressed pulp:				
Clarified with enzyme filtered	2.52	6.80	65.0	9.80
Not clarified, not filtered	5.70	10.00	60.3	8.37
Not clarified, filtered	2.26	7.8	61.5	8.68
Pectin from dried pulp:				
Clarified, filtered	3.06	8.60	62.2	8.18
Clarified, not filtered	4.33	8.40	60.6	8.22
Unclassified, filtered	3.70	9.60	59.3	7.92

Table 6.—Comparison of the chemical constituents of the acid- and alkaline-treated pectins.

Treatment	pH	Ash (%)	Galacturonic acid (%)	Arabinose (%)	Methoxyl content (%)
HCL	2.0	2.80	64.50	9.83	7.85
NaOH	7.6	5.72	61.50	7.44	5.70
NH <sub>3</sub>	8.0	3.58	72.30	6.26	6.67
NH <sub>3</sub>	9.0	3.80	75.90		5.59
NH <sub>3</sub>	11.0	3.03	67.45		2.10

than those treated with acid. When the pectin is treated with alkaline, partial deesterification takes place, yielding low methoxyl pectin (10). If the pectin solution stands from 5 to 6 hours at pH 9, the methoxyl content drops from 7.8%) to 5.70. At pH 11, the deesterification goes as far as 2.10 percent of methoxyl. The demethoxylation is not just a singular change in the molecule as Hills (13) and co-workers point out, but several reactions might take place which change the behavior of the resultant low methoxyl pectin.

**Purification of Pectin, Table 7.**—Twenty-five grams of crude pectin prepared from dried pulp was dissolved in 500 cc. of distilled water using a Waring blender for dispersing the pectin. It was clarified with active carbon and filtered through celite filter aid. Without concentrating it was precipitated with alcohol in the ratio of 1:2.5; the precipitate was washed twice with 80 percent alcohol making it acid with HCl to pH 3, and twice with 95 percent alcohol. Centrifugal separation was employed in place of filtration. A small

Table 7.—Purification of pectin; influence of reprecipitation on the composition.

Number of precipitations with alcohol	Ratio of water to alcohol	Ash (%)	Galacturonic acid (%)	Arabinose (%)	Methoxyl content (%)
0	1:2.5	5.97	67.5	12.37	7.75
1	1:2.5	5.00	67.9	9.62	8.52
2	1:2.5	4.26	68.3	6.64	8.68
3	1:2.5	3.51	70.8	6.50	9.27
4	1:2.5	2.85	69.1	8.70	9.00
5	1:1	1.41	71.0	8.30	9.96
Dialyzed and reprecipitated	1:1	0.90	76.0	7.30	10.70

quantity at each operation was washed with ether and saved for analysis. The same procedure was repeated up to the fourth precipitation. The fourth precipitation did not increase the methoxyl content and galacturonic acid any further. Upon changing the ratio of water and alcohol to 1:1, more ballast material was removed and the methoxyl and galacturonic acid content increased. It was then dialyzed against distilled water. We succeeded in reducing the ash during the purification process from 5.97 to 0.96. We shall attempt to de-ash the beet pectin by the use of ion-exchange agents (as it was done with citrus pectin) in order to further reduce the inorganic salt content.

As we see from the figures, the methoxyl went up from 7.75 to 10.70 and the galacturonic acid from 67.8 to 76.0.

**Physical Properties of Beet Pectin.**—The viscosity of aqueous solutions is one of the most significant properties specifying the quality of various pectins. In this respect all of the beet pectins prepared under acid conditions exhibited only minor variations, within relative values of 2.6 to 3.0 for one-half percent solutions at 25° C. Even repeated precipitation, coagulation at different alcohol strengths, etc., did not change the relative viscosity appreciably. Alkaline precipitated materials exhibited tremendous decreases in viscosity behavior as well as methoxyl contents. This is exhibited in table 8, which also includes typical values of fractions precipitated under different conditions. For comparative purposes the relative viscosities of typical citrus and apple pectins were ascertained to be 4.0 and 5.0 respectively.

Table 8.—Relative viscosities of pectin precipitation at different concentrations of alcohol.

Treatment			
pH	Precipitation with alcohol (%)	Relative viscosity 0.5-percent solution (25° C.)	Methoxyl content (%)
2	50	2.38	8.50
2	60	2.68	8.50
2	70	2.85	—
2	80	2.89	9.50
(reprec. 4 times)			
2	85	2.80	10.70
8	85	1.80	5.70
9	85	1.13	2.50
11-12	85	1.10	2.10

The relatively low viscosity values, as well as direct tests, confirm the general impression that ordinary sugar beet pectin is a non-jellifying type. In fact the best consistency obtained in our experiments was about equivalent to ordinary vaseline salve. This jell did not "cut" well.

It was observed qualitatively that several of the pectins produced exhibited a viscosity which seemed to increase tremendously upon acidifying. Since this is a characteristic of pectins capable of forming low sugar jells, we investigated these materials further although they did not meet the differentiating value of 0.5 in the degree of methoxylation (13). In no case was anything better than a very thick viscous liquor obtained. With a demethoxylated product (2.1 per cent OMe, 0.16 degree of methoxylation) the result was the same.

### Discussion

The sugar beet pectins prepared for this study are apparently of the same general chemical nature as those derived from fruit sources. This is obvious from examination of the galacturonic acid, arabinose, methoxyl content, etc. They meet and even exceed the minimum requirements set up by the National Formulary for Commercial pectins and can be purified to an extent suggested for pure pectins.

The National Formulary does not specify any gelling qualities for pectins since it is felt that this behavior is still a highly arbitrary matter. In this respect the beet pectins, as prepared at the present time, are different from those of other sources. The difference is apparently not one of chemical nature, since the beet pectins satisfy these requirements of reactivity. As first suggested by Schneider, the defect is to be influenced by a relatively lower molecular weight of the pectins derived from the beet. His representative values are:

Citrus pectin.....	150,000 to 220,000
Apple pectin.....	90,000 to 280,000
Beet pectin.....	20,000 to 25,000

The same relative order was confirmed in this work by means of viscosity measurements as presented in table 9. The absolute order of these values is also designated by the value of 43,000 to

Table 9.—Viscosity of pectin of different sources.

0.5 percent Solution	Relative viscosity at natural pH
Citrus 180 grade	4.0
Apple 237 grade	5.0
Crude beet pectin	2.5



46,000 ascertained through the courtesy of Dr. H. Owens of the Western Regional Laboratory, for the standard beet pectins prepared by us.

The possibility remains that this low molecular weight may be the consequence of the comparatively drastic treatment encountered in previous factory operations. However, we have made preliminary experiments indicating that beets, extracted under conditions found to be most favorable for the production of maximum quality of citrus pectins, yield a product of the same general nature and gelling quality as the product from pulp.

The question of the effect of pretreatment and extraction conditions in this property, as well as the possibility of increasing the molecular size of the derived product, is to be investigated further.

### Summary

The pectin in beet pulp was investigated by chemical and physical means.

The rate, time, temperature, pH, and concentration, as related to the extraction, were studied.

The pressed and dried pulp and the pectin prepared from both were analyzed and compared; no adverse effects of drying could be detected on this basis.

The effect of enzyme for clarification on pectin from pressed and dried pulp was studied in both cases; the beneficial effect of enzyme treatment was prevalent.

The alkaline treatment of pectin showed a decrease in methoxyl content.

Purification of pectin was carried out by repeated precipitation and subsequent dialysis. The increase in galacturonic acid and methoxyl content and decrease of ash was significant.

The physical properties—molecular weight, viscosity, and jelling properties were examined and discussed in connection with standard citrus pectin.

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### Literature Cited

1. Bauer and Link. *J. Biol. Chora.* 105: 1. 1934.
2. Corbeau, L. and Borgers, W. *Chem. Weekbl.* 1932.

3. Deroaux, G. Arch. Intern. Pharma. 62:100. 1939.
4. Ehrlich, F. and Schubert, F. Berichte, Deutsche Chemische Gesellschaft. 62:1974. 1929.
5. Ilirstand, E. L. and Jones, J. K. Chem. Society, London. 454. 1939.
6. Joseph, G. H. Bulletin of the National Formulary Committee. 9: No. 1 & 2. 1940.
7. Manville, I. A. and Deithel, F. J. Ind. Hyg. Toxicol. 22:35. 1940.
8. Mark, H. and Meyer, K. Per Aufbau dor hockmolekularen Natur Stoffe. 1930.
9. Molyoth, G. Klin, Wochenschrift. 13: 51. 1934.
10. Owens, H. S., McReady, R. M., and Mallay, W. D. Tnd. and Eng. Chem. 36: 9:36. 1944.
11. Pulmer, K. and Hartfog, M. J. A. C. S. 67: 2022. 1945.
12. Schneider, G. and Bock, H. Berichte, Deutsche Chemische Gesellschaft. 70B. 1617. 1937.
13. Speiser, R., Eddy, C. R., and Jills, C. H. Journal of Physical Chemistry. 49: 563. 1946.