

THE EXTRACTION AND PRECIPITATION OF PECTIN

Thesis

by

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Purpose of the Research

The research was undertaken in an effort to find an economical and efficient means of extracting pectin from orange peels and to find a method of precipitating the pectin and so obtaining it as a solid. A secondary or preparatory purpose was to find a convenient and accurate method of determining the amount of pectin present in a solution.

Theory

Pectin is known to be a colloid, but its exact composition is not known. Th. von Fellenburg¹ says that pectin is composed of a number of compounds and assigns the following composition to orange pectin: araban, 36.1%; methyl pentosan, 6%; galactan, 49.3%; and methyl alcohol, 11.5%. Colloids can be divided into two classes. The first of these imbibe and retain water and the colloidal particles contain much water. Sols of such colloids may have high viscosities and are only precipitated with difficulty. Such colloids are called hydrophilic. Those of the second class are termed hydrophobic and are substances to which can be ascribed no special relationship with water. A sol of a hydrophobic colloid shows properties very little different from those of the dispersion medium. The viscosity of such sols is, for example, very little different from that of water. Hydrophobic sols are very easily precipitated by the addition of an electrolyte or by properly adjusting the H^+ ion concentration.

¹J. C. S., A, 1915 i - 774.

Hydrophilic compounds are, on the other hand, not precipitated by electrolytes and not easily precipitated by adjusting the H^+ concentration. The following experimental work shows pectin to be^a hydrophilic compound. Sols of hydrophilic colloids can be precipitated by and with other colloids of opposite charge. Pectin is a negative colloid and as such it is to be expected that it will be precipitated by a positive colloid. This is found to be the case, as will be shown below. When one colloid is precipitated with another, the two are so closely intermingled as to make complete separation extremely difficult. This is particularly true in the case of pectin precipitates as solvents in which the precipitating colloid is soluble make a gel of the pectin. This applies particularly to aqueous solvents.

Previous Work

Pectin has been sold for some years, but has principally been marketed as a solution rather than as the solid. The advantages of solid pectin over the solution are numerous, including reduced cost of packing and shipping, and the possibility of using small amounts from a large container without danger of the remainder of the lot spoiling. By far the greater part of the pectin of commerce is at present produced from non-citrus fruits, berries and apples being the principal sources. There is one company, the Exchange Lemon Products Co., located at Corona, California, which is producing dry pectin from citrus fruit peels. The pectin plant is operated in conjunction with a citric acid and aromatic oil plant. The extraction

is carried out using a dilute acid solution as the extracting medium and the precipitation is effected by the double colloid method, using $\text{Al}(\text{OH})_3$, precipitated from an AlCl_3 solution as the precipitating colloid. The precipitated colloid mixture is then treated with alcoholic HCl to remove the $\text{Al}(\text{OH})_3$. This removal is not complete, tests made on the product showing around 10% $\text{Al}(\text{OH})_3$ in it. The alcoholic HCl washing is, in addition, rather expensive and not very convenient. The water concentration must be very low to avoid forming a gel of the pectin and thus making it unhandleable. This makes it necessary to use either low HCl concentration or to use gaseous HCl to further saturate the alcohol without adding more water. AlCl_3 is, moreover, not very soluble in a water-alcohol mixture which adds to the difficulty of effecting complete washing.

Some experimental work has been done to determine a satisfactory method of determining the amount of pectin present in a solution. The four general methods which have been used for quantitative determinations of pectin are: precipitation of pectin with $\text{C}_2\text{H}_5\text{OH}$, precipitation as calcium pectate, determination of viscosity of solution and taking this as a measure of the pectin present, and determining the gelling power of the pectin present by forming a gel with sugar. Each of these methods possesses some disadvantages. The alcoholic precipitation is said to be inaccurate and the results of Frederick Hardy² and Haynes and Carré³ show this to be the case. The calcium pectate precipitation method, developed by Haynes and Carré³ is apparently accurate, but it possesses the disadvantage of being a

²Frederick Hardy, *Biochem. Journ.*, Vol. XVIII, no. 2 (1924).

³Haynes and Carré, *Biochem. Journ.*, Vol. XVI, pg. 60-69 (1922).

very involved procedure and requiring a long time to carry out. The minimum possible time is 15 hours and the recommended time 2 days.

The viscosity of a solution is not a true measure of the amount of pectin present therein. In the first place most solutions to be tested contain other substances which affect their viscosities. Secondly pectin, being in colloidal suspension rather than in true solution, does not govern the viscosity of its solutions in the manner in which a true solute would. Thirdly, unless a special form of viscosity pipette is used, the results are found to be absolutely worthless; due to turbulent rather than viscous flow in the stem of the pipette. Frederick Hardy² has proved the invalidity of viscosity measurements taken with an ordinary pipette.

Inasmuch as the value of pectin lies in its power of gel formation the ~~direct~~ direct measurement of this power should be the best way of estimating the amount of pectin present in a solution. This method was used by Johnstin and Denton⁴. The difficulties encountered in attempting to use this method are outlined under experimental work.

The extraction of pectin from the orange peel is shown to be best carried out in an acid solution. F. Hardy² made a number of test runs, using various concentrations of HCl and citric acid, ranging from .002 N. to 1.5 N. He obtained the best results by the use of a 0.25 N. citric acid solution, carrying on the extraction for 20 minutes at 98°C, then allowing the mixture to cool for 10 minutes. Hardy's results also indicate that complete pectin recovery can not be expected in one extraction, the first treatment

⁴Johnstin and Denton, Ind. Eng. Chem., 15, 780 (1923).

removing slightly over 50% of the extractable pectin from the peel and each succeeding treatment removing about 50% of the extractable pectin left by the preceding extraction. The results of Hardy are particularly valuable as they are quite extensive and as the amount of pectin present in each extract was determined in three ways - by alcoholic precipitation, by precipitation as calcium pectate, and by viscosity measurements.

Experimental Work

It was first desired to determine the best means of estimating the amount of pectin present in a solution. As stated above, a direct measurement of the gelling power seemed to be the best way of determining the value of a solution of pectin, so this method was tried. If it were possible to determine the relative rigidities of a series of gels made from pectin solutions and sugar, this should prove to be a very satisfactory way of determining the relative gelling powers of a series of pectin solutions. A viscosimeter is clearly unsuited for this purpose, as once the structure of the gel is broken its viscosity means little.

It was suggested by Dr. Lacey that it might be possible to determine the relative hardness or resistance to penetration of a series of gels by the use of an instrument similar to the penetrometer used for asphalt testing. Acting on this suggestion an effort was made to adopt a penetrometer to gel testing. The needle used in asphalt testing is, of course, far too small in cross-section to give any reading as it drops through the most rigid gels with almost free fall velocity. A plunger of greater cross-section area was tried, but found unsatis-

factory, due to the "skin" which forms at the surface of the gel. Even when this "skin" is removed a plunger of large enough cross-section to move with a measurable velocity does not move at a uniform speed when penetrating the gel. The motion when it is penetrating the upper layers is slower than when penetrating the bottom layers, in spite of the increased side friction. As the penetration tests were made on jellies which were about 20 hours old the variation of rigidity in the different layers can not be ascribed to incomplete "setting" of the jelly due to lack of time.

Believing that a penetrometer test could not be used satisfactorily to determine gel strength, it was decided to make a standard test series of gels from known quantities of pectin and sugar in such a way that a sample gel could be made under the same conditions from a pectin solution whose strength it was desired to ascertain. The sample gel would then be compared to the standard test series and it would be considered to contain the same amount of pectin as the standard gel it most resembled in consistency. The pectin used for making the test gels was purified commercial pectin. The commercial product, before purification showed over 10% $\text{Al}(\text{OH})_3$ by combustion analysis. This commercial product was washed repeatedly with HCl - $\text{C}_2\text{H}_5\text{OH}$ mixture, the most efficient mixture containing 15% concentrated hydrochloric acid and 85% ethyl alcohol. Solutions stronger in HCl could not be used because the water present partially dissolved the pectin making a gel which, of course, could not be washed. After many washings with the above mixture, the Al salt content of the pectin was reduced to 3.1%, but could not be brought below this figure.

This shows one of the disadvantages of the double colloid precipitation method by which this commercial pectin was produced. The gels were made from this purified pectin as follows. The amount of pectin used for each gel was dissolved in 20 cc. of .05 N. citric acid. To this solution was added 12 grams of sugar, the sugar dissolved and the mixture boiled until a boiling point of 105°C was reached. The solution was then poured into molds and allowed to stand for 20 hours when the rigidity or texture of the resulting gels was noted and recorded. Table I gives the results for a series of standard gels.

Table I

Sample no.	Grams pectin	Relative viscosity	Rel. viscosity divided by grams pectin.	Consistency of gel after 20 hours.
1	1.0	All pectin did not dissolve.		
2	0.5	6.64	13.3	elastic solid
3	0.2	3.09	15.5	viscous solid
4	0.15	2.12	14.2	semi-solid; would not pour.
5	0.14	2.03	14.5	just pour
6	0.13	2.01	15.5	very viscous
7	0.1	1.72	17.2	entirely liquid
8	0.08	1.58	19.8	
9	0.06	1.42	23.7	
10	0.02	1.17	58.5	

The relative viscosities given in the third column are the viscosities of the pectin solution before the sugar was added. Columns 3 and 4 are given to show that the viscosity of a pectin solution is not a direct measure of the amount of pectin present even though no other viscous materials be present. If the pectin present were in direct ratio to the viscosity, the figures of column 4 would have a constant value. In such a series of gels only a few are of value, that is to say, only a few have a consistency which can be definitely fixed and which is proportional to the amount of pectin present. For example, if a gel is an elastic solid its consistency will apparently be the same even though a large excess of pectin over the amount needed to make the gel an elastic solid be present. Similarly, if the mixture is a liquid we only know, under the conditions of this test, that there is less than 0.1 grams of pectin present in 20 cc. of the solution. Only those gels whose consistency is on the borderline between solid and liquid can be referred to. A further difficulty arises here. The consistency of the gels changes on standing, even after the first 20 hours, so that either the tester must remember the exact consistency of each of the sample gels, or make up a new series each time it is desired to test some solutions for pectin; or, in the event that tests are being run continuously, make new standards at least once daily.

Possessing what appeared to be a fairly satisfactory method of determining the pectin present in solutions, experimental extraction runs were made. The first extractions were made using 0.25 N. and 0.1 N. citric acid as the extracting liquid. In each case 50 gms. of

fresh peel was extracted with 100 cc. of the citric acid solution, the peel being cut into strips roughly 1/8" wide before extraction and the extraction being carried out for 20 minutes at 98°C. The results of this run are given in Table II.

Table II

Sample no.	% Extract in sol'n.*	Normality of extracting acid.	Std. gel to which sample corresponds	Indicated % pectin in extract.
1	100	0.25	no. 2	2.5
2	75	0.25	" 3	1.35
3	50	0.25	liquid	-
4	25	0.25	"	-
5	100	0.13	no. 3	1.0
6	75	0.10	liquid	-
7	50	0.10	"	-
8	25	0.10	"	-

The results of this run show the higher acid concentration to be the best and also show the inaccuracy of the method used for determining the pectin concentration. The figures in column 5 for samples 1 and 2 should agree, but do not do so showing that, although gels were prepared under identical conditions, as far as possible, and carefully compared to the standard gels, a perfect or even a close determination did not result.

*The extract was diluted as shown in column 2 to make it possible to get a series of gels from each extract.

Since the extraction had been so thoroughly investigated by Hardy and since the time allotted for the research was rapidly passing, it was thought advisable to take the extraction outlined above, using 0.25 N citric acid, as the standard procedure and to pass on to the more interesting and less thoroughly investigated problem of precipitation. As mentioned above, under "Theory", pectin is a negative colloid and, as such, it was thought that it might be precipitated by the simple addition of an electrolyte. If such a precipitation were possible, it should be brought about more readily by an electrolyte containing a polyvalent positive ion than by one containing a monovalent positive ion. It would be convenient if the salt were soluble in C_2H_5OH so that this could be used to wash the salt from the precipitated pectin. Solutions of various strengths of the following salts were used: $FeCl_3$, $AlCl_3$, and $NiCl_2$, but the pectin was not precipitated.

Some colloids are sensitive to changes in the H^+ ion concentration so pectin solutions were titrated very slowly with HCl and NaOH solutions in an endeavor to find a H^+ ion concentration which would bring about the precipitation. No such concentration was found, the only precipitation taking place in a NaOH solution of considerable strength, the precipitate being dark in color and apparently not possessing the properties of pectin. The non-precipitation of positive ion electrolytes and by H^+ ion adjustment substantiates the theory that pectin is a hydrophilic colloid.

Inasmuch as the precipitation could not be successfully made by means of single salts or by the adjustment of the H^+ ion, it was decided to investigate the possibilities of commercial alcoholic

precipitation. The double colloid precipitation was rejected because of the difficulty of separating the colloid mixture resulting from such a precipitation. In order to produce a precipitate that will be completely dissolved when it is desired to use it, it is essential that the solution from which the precipitation is to be made be perfectly clear. It is only by filtering that a clear solution can be obtained; for the small particles of pulp which are suspended in the solution extracted from the peels are of such a density that they will not settle out from the solution, even after prolonged standing. In connection with this filtration the advisability of using filtercel, a purified form of diatomaceous earth, was investigated. The first results of this investigation are given in Table III.

Table III

No.	Acid used	Precoated filter paper.	Rel. viscosity of filtrate	Condition of filt.	Wt. of C ₂ H ₅ OH ppt. from 10 cc. extract
1	0.25 N. citric	yes	6.5	clear, light in color	0.12 grams
2	"	no	21.4	cloudy, light in color	0.28 "
3	0.25 N. HCl	no	9.65	cloudy, dark in color	0.41 "

The paper for sample 1 was precoated with filtercel to a depth of one to two millimeters. All viscosities were obtained by the use of a viscosity pipette, having a squat bulb and a long capillary stem. The alcoholic precipitates were obtained by adding the filtrate to an equal amount of alcohol, the addition of further alcohol producing no

further precipitate. The data of this table indicates that the filter-cell adsorbs a considerable portion of the pectin but as only a relatively small amount of the solution was run through the filter, these results must not be given too much weight. This table also shows that HCl, while giving a larger alcoholic precipitate than citric acid, is not suitable because the filtrate and alcoholic precipitate are very dark in color. The extract filtered through the precoated filter less rapidly than through the filter which was not precoated. Further tests gave the same results.

The proper amount of alcohol to be used to insure complete precipitation was determined in the following manner. To a number of 10 cc. samples of the unfiltered extract, obtained by the method which was adopted as standard for extraction, were added various amounts of C_2H_5OH and the precipitate filtered out. Further C_2H_5OH was then added to the filtrates and the filtrate originally containing the smallest amount of C_2H_5OH which did not become cloudy on the further addition of C_2H_5OH was considered as having the proper amount of C_2H_5OH . By this procedure it was found that equal volumes of extract and alcohol produced complete precipitation without the use of excess alcohol. As the precipitation is dependent on alcoholic concentration the amount of pectin present does not seem to change the ratio of H_2O and C_2H_5OH necessary to produce complete precipitation.

The alcohol precipitate was somewhat gelatinous in character, but if precipitated cold by a sufficient quantity of C_2H_5OH it was easily separated from the liquid by filtration. If, however, it was

precipitated hot or by an insufficient quantity of C_2H_5OH it was extremely difficult to handle. It was found that a temperature of above $50^{\circ}C$ could not be used in drying the precipitated pectin without danger of it turning dark in color.

Paper not being practical for a commercial filtering machine tests were run using duck as the filtering medium. 260 grams of fresh orange peels were heated with 520 cc. of 0.25 N. citric acid for 20 minutes at $98^{\circ}C$, on a water bath. The solution was decanted and drained from the large pieces of peel and divided into three samples, each of 75 cc., which were treated as follows:

Sample 1. Filtered through precoated duck after admixture of 1 gram of filtercel.

Alcohol precipitate - 0.22 gram

Filtrate clear.

Filtered easily.

Sample 2. Filtered through precoated duck, no admixture of filtercel.

Filtrate clear.

Filtered with considerable difficulty.

Alcoholic precipitate - 0.29 gram.

Sample 3. Filtered through plain duck.

Filtrate cloudy, but cleared up by repeated filtration through same filter.

Filtered very easily.

Alcohol precipitate - 0.35 gram.

These results reaffirm the adsorption of pectin by filtercel and also show that its use is not essential to a clear product.

Some tests on the advisability of running a second extraction on the peels and which showed that this was highly desirable concluded the laboratory work.

Conclusions

Pectin, or as it is sometimes called, pectinogen, is a negative hydrophilic colloid whose sol is not sensitive towards hydrogen ion concentration. It can best be extracted from orange peels by the use of a 0.25 N. solution of citric acid and can be precipitated from this solution by the addition of a quantity of C_2H_5OH equal in volume to the extract. The extract should be clarified by filtration through duck before the precipitation is made. The use of filtercel or similar substances is not recommended, as they adsorb considerable quantities of pectin. The alcoholic precipitation of pectin should be commercially practicable, if it is put on a large enough scale to make possible the use of efficient machinery, solvent-recovery driers, and rectifying stills for the alcohol used. Of the methods proposed for the estimation of the amount of pectin in a solution only the calcium pectate precipitation method is accurate and it takes too long to be of great use.

I wish to thank Dr. W. N. Lacey under whose direction this work was carried out, for the suggestion to undertake the work and for the suggestions he offered during the progress of the investigation.

Summary

An effort was made to find a method to commercially produce pure pectin. Extraction by means of 0.25 N. citric acid solution heated to 98°C for 20 minutes in contact with the cut-up orange peels was found to be satisfactory, but for good yield the extraction process should be repeated. The extract can be filtered through duck to give a clear solution. Precipitation can be carried out by the addition of an equal volume of C₂H₅OH to the clarified extract. The precipitate is easily filtered and must be dried at a low temperature. The disadvantages of the various methods of pectin estimation are pointed out. Pectin can not be precipitated by the addition of electrolytes or by H⁺ ion concentration adjustment. Pectin precipitated with a positive colloid can not be completely freed from this precipitating colloid.

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