CLXXVII. THE PECTIC SUBSTANCES OF PLANTS

VI. THE RELATION BETWEEN JELLY STRENGTH, VISCOSITY AND COMPOSITION OF VARIOUS PECTINS

BY ERNEST WALTON BENNISON
AND FREDERICK WALTER NORRIS

From the Department of Industrial Fermentation, University of Birmingham

(Received 15 July 1939)

THE conditions of formation of pectin-sugar-acid jellies have been exhaustively studied in recent times, notably by Tarr [1923; 1926] and Tarr & Baker [1924] in America, and by Ogg [1924] in Great Britain, and the principles laid down have been extended in lengthy investigations by Myers & Baker [1926-34]. In spite, however, of a mass of data obtained and analysed by the latter workers with reference to the relation between composition and jelly strength, the problem is still an open one.

Many workers have insisted that an adequate methoxyl content is a prerequisite of jelly formation by a pectin and that methoxyl content may be used as a criterion of jelly strength. These beliefs are denied by Myers & Baker who find that methoxyl content is not a measure of jelly strength: the power of forming jellies may be completely destroyed without loss of methoxyl and conversely the methoxyl content may be considerably lowered without appreciable loss of jelly strength.

Myers & Baker found that no single constituent of the pectin complex was a criterion of jelly power. Recent views on pectin constitution have replaced those on which they based their arguments, but they made the important suggestion that jelly power was a function of the degree of polymerization of the galacturonic acid. The work of Schneider & Henglein [1936], Schneider & Bock [1937] and Schneider & Fritschi [1937], Hirst & Jones [1938; 1939], Beaven & Jones [1939] and of Smith [1939] indicates that the essential part of the pectin complex consists of a long chain of galacturonic acid units, the araban and galactan usually found in most preparations being regarded as adsorbed non-essential constituents. Since viscosity is a measure of molecular size and since viscosity and jelly strength are related, the suggestion of Myers & Baker may be restated in the form that jelly strength is a function of molecular size, or of chain length of the essential constituent.

In the present investigation an attempt has been made to correlate jelly strength with viscosity, and chemical composition with both, in the case of pectins prepared from different sources by different methods. Artificial esters produced by methylation of pectic products have also been employed in the course of the study. The views of Myers & Baker, who used pectin from one source, prepared by the same method throughout, have been largely confirmed for the more general case indicated.

EXPERIMENTAL

Preparation of materials

Pectolic acid. This product was originally prepared by Ehrlich & Schubert [1929] and by Ehrlich & Guttmann [1933] and their methods were employed in this instance with but slight modification.

50 g. of commercial citrus pectin were gradually added with rapid mechanical stirring to 750 ml. of 5 % HCl in a bolt-head flask maintained at 70–75°. After heating for 1½ hr. precipitation of the flocculent voluminous pectolic acid was complete. The precipitate was filtered off when cool, washed first with 2 % HCl and then with water to the point of solution. After boiling with 95 % alcohol for 30 min., the precipitate was air-dried. Purification was effected by dissolving 20 g. in 2 l. of boiling water, cooling in the refrigerator and adding 120 ml. of conc. HCl. The precipitate was filtered off, washed in dilute HCl, then water and finally 50 % alcohol until all trace of acid was removed. It was dried in graded strengths of alcohol, then *in vacuo* and finally in a Fischer drier at 78° using P₂O₅. The final product was a white powder, free from ash, and contained 94.24 % anhydrogalacturonide.

Methyl pectolate and pectate. Buston & Nanji [1932] described a method for the preparation of methyl esters of pectic acid by treating the Ag salt with CH₃I under pressure. The elimination of the colloidal AgI formed is a difficulty which these workers overcame by warming with a little KBr. In preliminary experiments we found that satisfactory coagulation of the AgI was still difficult and other means of separation were sought, including saturation with quinine hydrochloride, or filtration through kieselguhr. No modification was entirely satisfactory but it was found that a larger excess of CH₃I produced the AgI in a form which could be readily filtered or centrifuged.

The method finally adopted was as follows: 2 g. of Ag pectate or pectolate were heated at 90° for 16 hr. in a pressure bottle with 25 g. CH₃I in 30 ml. CH₃OH. The mixture was then poured into 250–300 ml. of water and heated to 97° for a few minutes. The liquid was allowed to cool and filtered through paper pulp; if the filtrate was not clear at this stage it was centrifuged. The clear filtrate was precipitated with alcohol, the slimy precipitate being separated and dried as usual.

Pectins. Pectins were prepared from orange peel, apple pomace and beet pulp by methods which have been described in previous communications (see especially, Norris & Resch [1937]). Samples were prepared from these sources by: (a) ammonium oxalate extraction [Norris & Schryver, 1925]; (b) autoclave extraction [Ehrlich, 1932; Ogg, 1934]; (b 20 and b 40) autoclave extraction in presence of 20 and 40 % sucrose; (c) H₂SO₄ (N/10) extraction [Food Manufacture, 1936]; (d) HCl extraction at pH 1.4 and 60° [Myers & Baker, 1929; 1931]; (d 40) HCl extraction at pH 1.4 and 60° in presence of 40 % sucrose.

These methods do not require further comment at this point.

Experimental methods

Determination of jelly strength. A slightly modified form of the pectinometer described by Buston & Nanji [1932] was used. It consisted essentially of a pan on to which weights could be loaded, connected over pulley wheels to a plunger in a cylinder which contained the jelly. The movement of the plunger through the jelly with steadily increasing weights in the pan was indicated by a pointer which magnified the movement considerably. All parts coming into contact with the jelly were made of stainless steel.

A standard procedure for preparing the jellies was adopted: the requisite weight of pectin (0.10 or 0.20 g.) was dissolved in 10 ml. of 0.2% tartaric acid with 6 g. of sucrose in a 30 ml. beaker. The mixture was evaporated to 10 g. by boiling, and the hot liquid poured into the container of the pectinometer and allowed to set at 13°. Preliminary experiments had shown that jelly strength was constant after 20 hr., and 20–24 hr. were allowed for setting before taking strength readings. The strength is simply recorded as the weight in the pan required to give a predetermined definite movement of the plunger in the jelly.

Determination of viscosity. The viscosity was determined by means of Ostwald type viscometers maintained at 25° in a constant temperature water bath. In all cases a 0.5% solution (on a moisture-free basis) of the product was used.

Analysis of products. The analytical methods adopted were as given by Norris & Resch [1937]. It may be pointed out, however, that the results given for "anhydrogalactose" are difference figures and pending actual determination of galactose must be treated with reserve.

Jelly strength of methyl esters of pectolic and pectic acids

Short of complete degradation to galacturonic acid, pectolic acid appears to be the simplest derivative of pectin. Since a number of workers have suggested that methoxyl groups are a desirable, if not essential requirement for jelly formation, the jelly strength of methyl pectolate was investigated. A typical sample had the composition: uronic anhydride, 85.64%; methoxyl, 13.37%; but in no case did such a sample yield a jelly.

The methyl esters of pectic acids were next investigated. The products from beet gave no jelly even in cases where the product contained as much as 11.7% methoxyl. In one case only of samples from orange peel was a jelly obtained and this was of very poor strength, not comparable with that of orange peel pectins. Again, with the ester of pectic acid derived from commercial citrus pectin of high jelly strength, no samples were obtained which gave jellies. This in spite of the fact that the esterification was carried out under the most gentle conditions, and all details of preparation were arranged in accordance with what experience had indicated to be the best way to ensure maximum jelly strength in the product. For comparison, the analysis of a typical pectin ester and the commercial pectin from which it was obtained via the pectic acid, are given:

	Uronic %	Anhydro- galactose %	Anhydro- arabinose %	Methoxyl %	Ash %	Jelly strength g.
Ester	84.60	1.28	1.04	13.08	0.33	0
Pectin	86.56	4.14	0.91	8.39	3.14	207 (1% jelly)

It is thus obvious that methoxyl content plays no essential part in jelly formation. However, a certain degree of esterification is necessary if the product is to be readily soluble in water, and in this limited sense methoxyl is a requisite of jelly formation.

As a result of esterification there is no radical change in the composition of the products but some change must occur which prevents jelly formation. This may possibly be a resolution of the complex into components which are then co-precipitated by alcohol, or, more probably, a disaggregation of the polygalacturonide into smaller units.

Jelly strength of pectins

As a preliminary to jelly strength tests on prepared pectins, the effect of the drying treatment was first examined. A number of samples of the same commercial citrus pectin were dried for varying periods in an oven at 98° and the jelly strength then determined for 1 % jellies, moisture content being allowed for. The results are shown in Fig. 1.

Fig. 1.

"Relative viscosity" is discussed later. After several days' heating, no jelly could be obtained.

There is thus a very serious loss in jelly strength on heating. Drying at 78° in a Fischer drier for 48 hr. involved no measurable loss in jelly strength, but in order to obviate any possible source of error heat-drying was abandoned, the products being dried in a vacuum desiccator at room temperature.

Table I shows the jelly strength of prepared pectins in relation to their source and mode of preparation. In this and subsequent tables jelly strength is given as g. for a 2% jelly.

Table I

Method of preparation	Orange peel		Apple	Beet
	I	II		
Ammonium oxalate (a)	230	122	117	Nil
Autoclave (b)	Nil	Nil	Nil	—
Autoclave + 20 % sucrose (b 20)	—	Nil	Nil	—
Autoclave + 40 % sucrose (b 40)	—	Nil	Nil	—
H ₂ SO ₄ (c)	—	—	—	Nil
HCl; pH 1.4; 60° (d)	280	330	107	Nil
HCl; pH 1.4; 80° + 40 % sucrose (d 40)	—	315	—	—

Remarkably few of the pectins obtained yielded jellies at 2% concentration, and it may be noted that since it is unusual for investigators of pectin to determine the jelly power of their products, it is probable that in many instances such products did not possess the essential jellying characteristic of pectin.

Two facts are at once observable from the table: first, no beet pectin gave a jelly, a fact fairly generally recognized. It is of interest to point out that Ehrlich [1932] used "pectins" from beet in his researches. The only claim to the production of jellies from beet pectin of which the authors are aware is contained in an article [*Food Manufacture*, 1936] which mentions jellies from beet pectin prepared by 40 hr. extraction at 60° with $N/10$ H_2SO_4 .

Secondly, no pectin prepared by autoclave extraction would yield a jelly. Tarr [1923; 1926] had found that boiling a pectin in presence of acid decreased the jelly strength, but that if sucrose were present the boiling period could be considerably prolonged without any deleterious effect. It was thought that a similar protective effect might be exhibited during the extraction of pectins by autoclaving or with acid; hence the use of 20 and 40 % sucrose in some of our preparations. The addition of these large amounts of sucrose had no harmful effect on the extraction and preparation but did not prevent loss of jelly power of the products.

Table II

Source	Method of preparation	Urone %	Anhydro-arabinose %	Anhydro-galactose %	Methoxyl %	Jelly strength g.
Orange peel II	(d)	75.52	4.89	7.23	12.36	330
Orange peel II	(d 40)	78.92	4.27	6.04	10.77	315
Orange peel I	(d)	73.20	7.76	10.07	8.97	280
Orange peel I	(a)	69.32	13.87	8.57	8.24	230
Orange peel II	(a)	76.64	9.09	5.56	8.71	122
Apple	(a)	70.72	9.71	9.73	9.84	117
Apple	(d)	67.72	5.39	16.83	10.06	107
Beet	(d)	56.12	17.44	17.79	8.47	Nil
Beet	(d)	50.36	15.38	26.38	7.88	Nil
Beet	(a)	59.24	21.60	9.55	9.61	Nil
Beet	(a)	59.16	16.45	14.89	9.50	Nil
Beet	(a)	57.28	22.69	12.89	7.24	Nil
Beet	(a)	51.84	25.02	15.69	7.45	Nil
Beet	(c)	66.04	9.19	18.54	6.18	Nil
Orange peel I	(b)	60.12	14.23	16.81	8.84	Nil
Orange peel II	(b)	72.12	8.87	7.42	11.59	Nil
Orange peel II	(b 20)	70.56	13.68	7.18	8.58	Nil
Orange peel II	(b 40)	74.52	9.39	4.63	11.46	Nil
Apple	(b)	64.54	6.15	19.32	9.99	Nil
Apple	(b 20)	62.27	6.92	20.85	9.96	Nil
Apple	(b 40)	62.73	6.78	20.64	9.85	Nil

Table II shows the complete analysis of products, from which it may be observed that the relation between chemical composition and jelly power is vague at best. No definite generalizations can be made, but certain points of interest may be indicated.

There is no direct relation between urone content and jelly strength. With the exception, however, of orange peel II pectins prepared by methods (b), (b 20) and (b 40) which are anomalous throughout, it will be seen that a high urone content favours jelly formation and conversely a high content of non-urone constituents—arabinose and galactose—inhibits jelly formation. Thus, apart from the anomalies mentioned, no pectin with less than about 68 % urone gave a jelly. In considering chemical composition and jelly formation it may be mentioned that the most satisfactory sample in regard to jelly strength was a commercial sample of citrus pectin which had a urone content of 86.56 %, an arabinose content of less than 1 % and gave a jelly strength of 207 g. for a 1 % jelly.

Reference to the methoxyl contents shows that there is no relation between jelly power and methoxyl content as previously indicated.

Viscosity relationships

Myers & Baker [1926-34] have shown that jelly strength of a pectin is a function of the viscosity of the pectin solution from which the jelly is made, provided that the pectins are derived from the same source by the same method of preparation.

In what follows an attempt has been made to correlate jelly strength and viscosity, and viscosity and chemical composition for pectins from different sources prepared by different methods.

Preliminary observations revealed the important phenomenon that the viscosity of the solution decreased for some hours after its preparation. Owing to the time necessary to effect solution it was impossible to take readings earlier than 1 hr. after addition of the pectin to the water, but thereafter readings were taken at intervals as shown in Table III.

Table III

Time of standing hr.	Relative viscosity
1	7.46
2.25	7.15
3	7.07
5	6.98
26.33	6.83

A similar effect was observed with all pectins examined. Thus, for comparable viscosity readings the time of standing must be standardized, and in all subsequent measurements this time was fixed at 5 hr.

It has been shown previously that whilst heat treatment has a deleterious effect on jelly strength, no change in composition is observable. Heat treatment thus affords a simple method whereby changes in jelly strength and viscosity can be measured for the same pectin. Results of this type have been shown in Fig. 1.

The graph showing change in viscosity with time of heating is similar to that showing changes in jelly strength. It may be interpreted possibly as showing the result of hydrolytic cleavage of the pectin in the presence of a small amount of moisture. The moisture content was 4.01 % after 1 hr. and 1.10 % after 8 hr.

When viscosity is plotted against jelly strength a curve (Fig. 2) is obtained whose points, within the optimum range of the pectinometer (80-200 g.), lie in a straight line, indicating strict proportionality. The divergence of the curve from the straight line outside such range is due to experimental difficulties in measuring the strength of a very stiff jelly on the one hand and of a very syrupy jelly on the other.

Thus, proportionality between jelly strength and viscosity may hold for one particular pectin submitted to varying degrees of heat treatment, but it is by no means certain that the same holds for different pectins prepared by different methods. This is exemplified in Table IV.

Inspection of the first two columns of the table shows that there is no direct relationship but that in general there is a certain parallelism. The differences are largely due to wide variations in composition and previous history. It would seem that there is a limiting viscosity below which jelly formation does not take place, but at the same time the viscosity may be above this limit and the pectin still fail to give a jelly. An anomaly of this type is exemplified by the non-jellifying pectin having a relative viscosity of 3.16: this was a beet pectin prepared

by HCl extraction at pH 1.4 and 60°. Two explanations of this may be offered: (a) that in beet pectin the polyuronide molecule is not large enough for jelly

Fig. 2.

Table IV

Relative viscosity	Jelly strength 2 % pectin g.	Relative viscosity of non-jellying pectins
5.20	330	3.16
3.58	315	1.64
2.38	122	1.62
2.10	107	1.42
2.09	65	1.38
1.94	117	1.36
		1.35

formation and it is the other substances present which give the pectin as a whole the viscosity observed; (b) it is more likely, however, that although the polyuronide molecule is of sufficient size, as indicated by viscosity, the contaminating substances are present in sufficient quantity to prevent jelly formation. This pectin contained over 40 % of non-urone substances.

The relationship between viscosity and source and method of preparation of pectins is indicated in Table V.

Table V

Method of preparation	Orange peel II	Apple	Beet
Ammonium oxalate (a)	2.38	1.94	1.64
Autoclave (b)	1.42	1.35	—
Autoclave + 20 % sucrose (b 20)	—	1.38	—
Autoclave + 40 % sucrose (b 40)	1.62	1.36	—
HCl; pH 1.4; 60° (d)	5.20	2.10	3.16
HCl; pH 1.4; 60° + 40 % sucrose (d 40)	3.58	—	—

This table yields, for relative viscosity, similar information to that of Table I for jelly strength. In addition, viscosity provides a measure for those

solutions which failed to give a jelly. The highest viscosity obtainable by auto-claved preparations was 1.62, the majority averaging about 1.4. This indicates a degradation of the pectin by treatment at temperatures of over 100° to a more or less constant level—a fairly definite degree of depolymerization.

A further point of agreement with the findings for jelly strength is that all the pectins from orange peel have higher viscosities than those from apple. This is in keeping with recent observations by Svedberg & Gralen [1938] who find that in the fruit juices the mol. wts, determined by ultracentrifugal methods, approximate to 40,000–50,000 for orange pectin, and to 25,000–35,000 for apple pectin.

Relative viscosity and composition are shown in Table VI. Only the first five pectins gave jellies.

Table VI

Source	Method of preparation	Temp. of extraction ° C.	Urone %	Non-urone %	Methoxyl %	Relative viscosity 0.5 % pectin
Orange peel II	(d)	60	75.52	12.12	12.36	5.20
Orange peel II	(d 40)	60	78.92	10.31	10.77	3.58
Orange peel II	(a)	95	76.64	14.65	8.71	2.38
Apple	(d)	60	67.72	22.22	10.06	2.10
Apple	(a)	95	70.72	19.44	9.84	1.94
Beet	(a)	95	51.84	40.71	7.45	1.64
Orange peel II	(b 40)	110	74.52	14.02	11.46	1.62
Orange peel II	(b)	110	72.12	16.29	11.59	1.42
Apple	(b 20)	110	64.54	25.47	9.99	1.38
Apple	(b 40)	110	62.27	27.77	9.96	1.36
Apple	(b)	110	62.73	27.42	9.85	1.35
Beet	(d)	60	50.36	41.76	7.88	3.16

As in the case of jelly strength it is impossible to draw definite conclusions with regard to the relation of chemical composition and viscosity. The method of preparation and the material used are factors at least equal in importance to composition of the products.

That previous history is an outstanding factor in determining the possibility of jelly formation is again indicated by results for prepared methyl esters of pectolic and pectic acids. Two typical examples gave the following results:

	Urone %	Non-urone %	Methoxyl %	Relative viscosity	Jelly strength
Methyl ester of pectolic acid	85.64	—	13.37	1.27	Nil
Methyl ester of pectic acid	84.60	2.32	13.08	1.17	Nil

The low viscosity of these products corresponds with their lack of ability to form jellies. Nevertheless, if composition were the only factor determining jelly strength and viscosity, these examples might be expected to give high values since each show high urone, low non-urone and high methoxyl contents. It is evident that the laboratory process of esterification involves degradation of pectolic and pectic acids to products of smaller molecular size.

SUMMARY

1. There is little relation between chemical composition and jelly strength or viscosity of pectins. In general, a high urone content connotes satisfactory jelly strength.

2. Methoxyl content is not a criterion of jelly strength: esters of pectolic and pectic acids prepared by methylation failed to give jellies; the methoxyl contents of prepared pectins bore no relation to their power of forming jellies.

3. The method of preparation of pectins is a vital factor with respect to jelly strength and viscosity. Autoclave preparation in absence or presence of sucrose inhibits jelly formation.

4. Jelly strength and viscosity of the same pectin are closely related and show rapid decreases when the pectin is heated. Viscosity shows a decrease when solution is first effected, but becomes constant after five or more hours.

5. The power of pectin to form jellies depends to some extent on composition, but primarily on molecular size as indicated by viscosity. Any pretreatment which tends to disaggregate the polygalacturonide chain tends also to cause loss of jelly strength.

REFERENCES

- Beaven & Jones (1939). *Chem. Ind. Rev.* **58**, 363.
Buston & Nanji (1932). *Biochem. J.* **26**, 2090.
Ehrlich (1932). *Ber. dtsh. chem. Ges.* **65**, 352.
—— & Guttmann (1933). *Biochem. Z.* **259**, 100.
—— & Schubert (1929). *Ber. dtsh. chem. Ges.* **62**, 1974.
Food Manufacture (1936). **11**, 162 (U.S. Patent, 2,008,999).
Hirst & Jones (1938). *J. chem. Soc.* p. 496.
—— — (1939). *J. chem. Soc.* pp. 452, 454.
Morris (1933). *Principles of Fruit Preservation*. London.
Myers & Baker (1926). *Bull. Del. agric. Exp. Sta.* no. 144.
—— — (1927). *Bull. Del. agric. Exp. Sta.* no. 149.
—— — (1929). *Bull. Del. agric. Exp. Sta.* no. 160.
—— — (1931). *Bull. Del. agric. Exp. Sta.* no. 168.
—— — (1934). *Bull. Del. agric. Exp. Sta.* no. 187.
Norris & Resch (1937). *Biochem. J.* **31**, 1945.
—— & Schryver (1925). *Biochem. J.* **19**, 676.
Ogg (1934). *Dissertation*, Univ. Cambridge.
Schneider & Bock (1937). *Ber. dtsh. chem. Ges.* **70**, 1617.
—— & Fritschi (1937). *Ber. dtsh. chem. Ges.* **70**, 1611.
—— & Henglein (1936). *Ber. dtsh. chem. Ges.* **69**, 309.
Smith (1939). *Chem. Ind. Rev.* **58**, 363.
Svedberg & Gralen (1938). *Nature, Lond.*, **142**, 261.
Tarr (1923). *Bull. Del. agric. Exp. Sta.* no. 134.
—— (1926). *Bull. Del. agric. Exp. Sta.* no. 142.
—— & Baker (1924). *Bull. Del. agric. Exp. Sta.* no. 136.