145. THE QUANTITATIVE BASIS OF PECTIN JELLY FORMATION IN RELATION TO pH CONDITIONS

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INTRODUCTORY

VARIOUS factors which govern the jellying properties of fruit pectins have been studied and described by Hinton [1939]. In the present paper it is proposed to devote attention to factors which can be varied at will, such as the effects of the concentrations of pectin and of other soluble substances on the strength of the jelly; the effect of the group of conditions which may conveniently be summarized under the term pH ; and the effects of such a minor modification of the pectin molecule as the de-esterification resulting from the action of pectase or of alkali.

It will be shown that pectins may be studied to a considerable extent from an ordinary physico-chemical standpoint, taking into account in particular the properties of solubility and of electrolytic dissociation. Hinton [1939] suggested that they must be regarded as complex mixtures of carboxylic acids of variable composition. It is impossible at present to separate the molecular components; hence the properties cannot be dealt with stoichiometrically. They can, however, be treated in a statistical sense, and on this basis it is possible to establish much useful quantitative knowledge and to correlate this with the jellying behaviour.

The superficial connexion between pH and the strength exhibited by a pectin jelly has been familiar for some time [Hinton, 1939, p. 15]. As the pH of a jellying mixture is raised the strength of the jelly it forms diminishes (other conditions, such as total solids concentration, remaining unaltered). If the pH is raised sufficiently, the mixture fails to form a jelly; on the other hand, if acid is added the jelly increases in strength down to a pH at which the strength reaches a maximum.

Pectins in dilute solution may be expected to behave like other organic acids. Dissociation of the acid groups must occur, and the degree to which this occurs will be related to the dissociation constants of the successively dissociating acid groups. The physical state of the pectin—i.e. its degree of coherence or of dispersion as a hydrosol—is determined by the potential difference between the pectin and the solvent; this potential difference arises from the dissociation of the pectin or of its salts. If the potential is discharged by suppression of the dissociation, the coagulating tendency of the molecules is no longer hindered. Jellying may be regarded as a form of coagulation in which the coagulated particles form a continuous network [Hardy, 1900]. Hence the conditions for the formation of pectin jellies must embrace the dissociation behaviour of the pectin and of its salts.

The acid groups of the pectin, so long as they are entirely in the free condition, may be expected to be dissociated to a minor degree only; hence the

pectin is very little ionized, and coagulation or jellying can readily occur. When alkali is added in partial neutralization, the number of dissociated groups increases because of salt formation, and coagulation or jellying is inhibited. This mechanism therefore is capable of explaining why, as the pH rises, the jelly strength diminishes and ultimately disappears completely.

When acid (e.g. HCl) is added to a pectin already partly neutralized in this way, the process is reversed; the dissociation of the acid groups diminishes and jellying can again take place. The net effect of the addition of the alkali and the acid is virtually the addition of a small amount of a more or less inert salt (e.g. NaCl).

NEUTRALIZATION DATA AND DISSOCIATION "CONSTANTS" OF PECTINS

The dissociation of the acid groups was studied by means of partial neutralizations of pectins from which the metallic bases had been first eliminated. For the preparation of these pectins, a considerable excess of HCl was added to a dilute solution of the pectin, followed after a short time by sufficient acetone to precipitate the latter in the free acid state. One litre of 0.5 % pectin solution required 30–40 ml. of conc. HCl and 700 ml. acetone. The precipitated pectin was filtered off, washed with 70 % acetone, and put back into 2 l. 70 % acetone, in which it was allowed to soak for 2 or 3 days. The filtration and soaking were repeated 4 or 5 times, until a portion of the filtrate, after removal of the acetone, gave no opalescence with AgNO_3 . The pectin was finally washed with 98 % acetone, spread out in a dish, and dried in a warm room. It was dissolved to a paste by mixing with pure sucrose (10 g.) and thymol (0.07 g.) and then gradually adding water while stirring so as to give a 4 or 5 % solution of the pectin. By allowing to stand with frequent stirring, and in some cases moderate warming, a smooth paste was usually obtained in a day or two. The pastes were kept in airtight glass jars.

The free acidity, saponification value, alkalinity of ash and calcium pectate yield of the pastes were determined by the methods already described [Hinton, 1939, pp. 11, 27, 28, 33]. The pectins were sometimes free from alkaline ash; in other cases there was a trace of alkalinity, which was allowed for when calculating the results on the assumption of its being due to an initial partial neutralization of the pectin with alkali.

In determining the neutralization data, the concentration was kept constant in each series. A number of separate portions of the pectin paste were weighed out into beakers provided with stirring rods, and to each portion was added a suitable amount of water followed by dilute NaHCO_3 (or K_2CO_3) solution of known strength. The hydroxide was purposely not used in order to avoid the risk of local saponification. Thus a series of mixtures was obtained with increasing amounts of alkali, but with the same total weight in each. The water and alkali were well stirred in and the beakers covered and left until the following day, when the weights were finally adjusted with small additions of water. The pH values were determined with quinhydrone, a 0.4 % solution of cream of tartar ($\text{pH}=3.57$) being used as a reference buffer. Within the range covered it may be taken that the CO_2 liberated from the carbonate had no significant effect.

Table 1 gives the results obtained with pectins from orange, red currant and strawberry. The de-ashing treatment was less successful with the strawberry pectin. The jellying qualities of the orange and red currant pectins were good; of the strawberry, feeble.

The partial neutralizations were effected with NaHCO_3 .

Table 1. *Partial neutralization data and dissociation constants of pectins*

Analytical details of pectins:					
As NaOH % of calcium pectate yield					
	Free acidity	Saponification value	Alkalinity of ash	Equivalent weight	
Orange	4.43	12.61	0.02	899	
Red currant	4.99	12.62	0.00	802	
Strawberry	3.96	14.64	0.38	922	

No.	Concentration of pectin		Degree of neutralization	pH	K Dissociation "constant" $\times 10^{-4}$
	g. per 100 g. water	g. equiv. per 1000 g. water			
Orange:					
1	0.59	0.0066	0.004	2.85	4.0
2	0.59	0.0066	0.160	3.07	3.5
3	0.59	0.0066	0.315	3.31	3.1
4	0.59	0.0066	0.473	3.62	2.5
5	0.59	0.0066	0.627	3.97	2.0
6	1.08	0.0120	0.004	2.67	4.7
7	1.08	0.0120	0.160	2.91	4.4
8	1.08	0.0120	0.315	3.16	3.9
9	1.08	0.0120	0.473	3.48	3.3
10	1.54	0.0171	0.004	2.56	5.4
11	1.54	0.0171	0.160	2.81	5.1
12	1.54	0.0171	0.315	3.11	4.4
Red currant:					
1	0.26	0.0032	0.000	3.04	3.6
2	0.26	0.0032	0.159	3.21	3.3
3	0.26	0.0032	0.318	3.43	2.8
4	0.51	0.0064	0.000	2.83	4.5
5	0.51	0.0064	0.159	3.04	4.0
6	0.51	0.0064	0.318	3.28	3.5
7	0.51	0.0064	0.478	3.60	2.7
8	0.51	0.0064	0.636	3.93	2.2
9	0.94	0.0117	0.000	2.67	4.8
10	0.94	0.0117	0.159	2.91	4.4
11	0.94	0.0117	0.318	3.19	3.9
12	0.94	0.0117	0.478	3.52	3.1
13	1.34	0.0167	0.000	2.57	5.2
14	1.34	0.0167	0.159	2.86	4.4
15	1.34	0.0167	0.318	3.15	4.0
Strawberry:					
1	0.43	0.0047	0.088	3.01	4.1
2	0.43	0.0047	0.262	3.24	3.6
3	0.43	0.0047	0.436	3.53	2.9
4	0.43	0.0047	0.611	3.89	2.3

The last column of the table gives the values for the apparent dissociation "constant", calculated as for a monobasic acid in the usual way, on the assumption that the sodium salt was completely dissociated. Activity coefficients have been neglected.

The results for the three pectins show a general similarity. The dissociation "constant" diminishes with increasing degree of neutralization, as found by Briggs [1934] for arabic acid. This acid is closely related to the pectins [Pauli & Palmerich, 1937], but has not the complexity which pectins owe to their partial esterification. From the data of Briggs, the dissociation "constant" of arabic acid has values of the same order as those now found for pectin, and the decrease with increasing degree of neutralization is similar. Briggs ascribed this decrease to the fact that the acid groups with the greater tendency to dissociate are first

neutralized, the average dissociation constant of the remaining groups thus being lowered.

The value of K at a given degree of neutralization decreased with dilution. In the case of the red currant pectin, this dilution effect appears to diminish at the higher concentrations, as can be seen from Fig. 1. There appears to be an approach to a constant value for each degree of neutralization. Briggs found similar effects for arabic acid.

Fig. 1. Variation of dissociation "constant" with pectin concentration (red currant pectin). Degree of neutralization = 0, x; degree of neutralization = 0.159, o; degree of neutralization = 0.318, +.

A comparison of neutralization data with Na and K as neutralizing metals was made, a second sample of red currant pectin being used (Table 2). This was of interest in connexion with differences in the jelling behaviour in presence of Na or K salts, to be discussed later. NaHCO_3 and K_2CO_3 solutions were used for the neutralizations.

Table 2. *Partial neutralization data with sodium and potassium as neutralizing alkalis*

Red currant pectin No. 2

Analytical details of pectin: Free acidity 5.27% (as NaOH % of calcium pectate yield)

Saponification value 13.30% (as NaOH % of calcium pectate yield)

Alkalinity of ash 0.01% (as NaOH % of calcium pectate yield)

Equivalent weight 758

Concentration of pectin in each mixture = 0.118 g. equiv. per 1000 g. water

No.	Alkali used	Degree of neutralization	pH	$K \times 10^{-4}$
1	No addition	0.002	2.23	3.2
2	Na	0.152	2.73	3.7
3	"	0.271	3.00	3.9
4	"	0.384	3.23	3.8
5	"	0.482	3.42	3.6
6	No addition	0.002	2.24	3.1
7	K	0.137	2.70	3.6
8	"	0.242	2.95	3.8
9	"	0.373	3.21	3.8
10	"	0.451	3.35	3.7

The neutralization curves are identical for the two neutralizations.

The values of K in the earlier stages of neutralization do not follow the same course as in the previous experiments, being unexpectedly low. This may possibly be due to the high concentration of pectin in this series; in the earlier stages of neutralization some aggregation may have occurred, thereby lowering the effective concentration of undissociated acid groups.

In the later stages of neutralization, after passing through a maximum, the values of K tend to become more concordant with those for the pectins previously examined.

The effect of the heat treatment of a pectin on its dissociation was examined, a second sample of orange pectin being used. A solution of the pectin was divided into two portions, one of which was gently boiled under reflux for 1 hr., cooled, and the ash-free pectin prepared by precipitation with HCl and acetone and purification as already described. The ash-free pectin was also prepared from the unheated portion of the solution in the same way. Neutralization data for both are shown in Table 3.

Table 3. *Partial neutralization data for pectin before and after boiling for 1 hr.*

Orange pectin No. 2					
		Unboiled	Boiled		
Analytical details of pectin	{	Free acidity	3.93	4.20 (as NaOH % of calcium pectate yield)	
		Saponification value	13.33	13.68 (as NaOH % of calcium pectate yield)	
		Alkalinity of ash	0.05	0.04 (as NaOH % of calcium pectate yield)	
		Equivalent weight	1005	943	
Concentration of pectin					
No.		g. equiv. per 1000 g. water	Degree of neutralization	pH	K × 10 ⁻⁴
Unboiled:					
1		0.0062	0.011	2.88	3.8
2		0.0062	0.177	3.09	3.6
3		0.0062	0.343	3.34	3.3
4		0.0062	0.510	3.64	2.8
5		0.0062	0.674	4.00	2.2
Boiled:					
6		0.0071	0.010	2.81	4.6
7		0.0071	0.155	3.00	4.2
8		0.0071	0.299	3.22	3.8
9		0.0071	0.445	3.47	3.3
10		0.0071	0.588	3.75	2.8

There is a slight difference between the two sets of values for K which is rather greater than can be accounted for by the different concentrations, except perhaps in the later stages of neutralization.

In jelly tests with these pectins at pH 3, the comparative jelly strengths obtained were: unboiled, 8.1 units; boiled, 5.5 units. The loss of jelling power was less than is sometimes found; however, it was sufficient to show that the value of the dissociation "constant" of the acid groups is not materially affected by changes in the jelling power of the pectin.

The effect of partial de-esterification was studied in the case of a third orange pectin. Part of a solution of the pectin was treated with an amount of NaOH sufficient only for partial saponification. The pectin was then separated in the ash-free condition in the usual manner, and at the same time the remainder of the original pectin was separated similarly. The partial neutralization data are

given in Table 4. (The metallic constituents had not been removed so thoroughly as usual from these pectins, and allowance has been made for this as already described.)

Table 4. *Effect of partial de-esterification on the neutralization curve and dissociation of a pectin*

Orange pectin No. 3					
Analytical details of pectin	Original		Partly de- esterified		% of calcium pectate yield
	Free acidity	4.23	7.50 (as NaOH		
	Saponification value	13.91	10.35 (as NaOH		
	Alkalinity of ash	0.16	0.17 (as NaOH		
	Equivalent weight	911	522		
Concentration of pectin					
No.	g. per 100 g. water	g. equiv. per 1000 g. water	Degree of neutralization	pH	$K \times 10^{-4}$
Original:					
1	1.06	0.0116	0.036	2.67	6.0
2	1.06	0.0116	0.176	2.92	4.7
3	1.06	0.0116	0.396	3.35	3.6
4	1.06	0.0116	0.589	3.85	2.1
Partly de-esterified:					
5	1.06	0.0204	0.022	2.53	5.9
6	1.06	0.0204	0.245	3.00	4.2
7	1.06	0.0204	0.381	3.29	3.5
8	1.06	0.0204	0.512	3.60	2.8

The values of K , when plotted against degree of neutralization, lie on the same curve for the two pectins, in spite of the appreciable difference in the equivalent concentrations. This confirms the tendency noted earlier, in connexion with the red currant pectin of Table 1, for K to approach a constant value (for a given degree of neutralization) at higher concentrations.

The comparative uniformity of the dissociation properties of the various specimens and grades of pectin would appear to justify the view that the dissociation constant is a characteristic of the galacturonic acid unit in the molecule and not of any particular proportion or arrangement of esterified and unesterified units.

The dissociation of a pectin in presence of a second acid was examined by comparing the neutralization of a mixture of the two to a given pH with that of the second acid alone to the same pH. The following calculation applies to these conditions.

Let $[T]_P$ and $[T]_A$ be the total equivalent concentrations of the pectin and of the second acid;

$[Na]_1$ the equivalent concentration of alkali required to bring the H-ion concentration of the mixture to a given value $[H^+]$;

$[HP]$ and $[HA]$ the equivalent concentrations of undissociated pectin and of undissociated second acid at that value of $[H^+]$; and

$[Na]_2$ the equivalent concentration of alkali required to bring the second acid only (in the same concentration as in the mixture but in a separate solution) to the same H-ion concentration.

Then in the mixture, assuming complete dissociation of the alkali salts, with sufficient exactness

$$[T]_P + [T]_A = [HP] + [HA] + [Na]_1 + [H^+]. \quad \dots\dots(1)$$

In the solution of the second acid only

$$[T]_A = [HA] + [Na]_2 + [H^+]. \quad \text{.....(2)}$$

The values of $[HA]$ in (1) and (2) are the same if it be accepted that the dissociation constant of the second acid is unaffected by the pectin, and hence, subtracting (2) from (1),

$$[T]_P = [HP] + [Na]_1 - [Na]_2. \quad \text{.....(3)}$$

For the pectin, at the pH in question and for the concentration used,

$$K = \frac{[H^+] ([T]_P - [HP])}{[HP]}.$$

Hence from (3),

$$K = \frac{[H^+] ([Na]_1 - [Na]_2)}{[T]_P - ([Na]_1 - [Na]_2)}. \quad \text{.....(4)}$$

Table 5 shows the neutralization data obtained with mixtures of a red currant pectin and citric acid, and the parallel data for the citric acid only which provide the values of $[Na]_2$.

Table 5. *Dissociation of pectin in presence of citric acid*

Red currant pectin No. 3

Analytical details of pectin

Free acidity 5.33 % (as NaOH % of calcium pectate yield)
 Saponification value 12.44 % (as NaOH % of calcium pectate yield)
 Alkalinity of ash 0.00 % (as NaOH % of calcium pectate yield)
 Equivalent weight 750

Neutralization of citric acid only

g. equiv. per 1000 g. water			
Citric acid × 10 ⁻⁴	Alkali × 10 ⁻⁴		pH
67.0	0		3.00
67.0	10.2		3.33
67.0	20.5		3.85
134.0	0		2.80
134.0	20.5		3.20
134.0	41.0		3.78

Neutralization of mixtures of pectin and citric acid

g. equiv. per 1000 g. water			Equiv. conc. of alkali for same pH with citric acid only (by inter- polation)		[H ⁺] × 10 ⁻⁴	K (by equation (4)) × 10 ⁻⁴
Pectin × 10 ⁻⁴	Citric acid × 10 ⁻⁴	Alkali × 10 ⁻⁴	pH	× 10 ⁻⁴		
67.0	67.0	20.5	3.04	1.2	9.12	3.7
67.0	67.0	41.0	3.40	11.6	3.98	3.1
67.0	134.0	30.8	3.02	11.4	9.55	3.9
67.0	134.0	61.5	3.45	30.7	3.55	3.0

The values of K for the pectin in these mixtures are in reasonably good agreement with those for red currant pectin alone at the same concentration and pH (cf. Table 1). It appears therefore that the dissociation of the pectin is not materially altered in dilute solutions by the presence of another weak acid and its salt, assuming that the dissociation of this other acid is not altered by the pectin.

This is an important result, for it governs the case of pectin in admixture with other acids and salts such as are present in fruit juices and which therefore usually accompany the pectin in the process of jelling. Advantage has been taken of this in the next section in dealing with buffered jelling mixtures.

THE RELATION BETWEEN THE JELLYING OF PECTIN
AND ITS DISSOCIATION

Experiments were undertaken with the object of studying the jellying of pectin in the light of the data established for its dissociation.

It is familiar that in jellying mixtures containing pectin there is, under given conditions of pH and total solids concentration, a minimum concentration of pectin which must be present for jellying to occur [Hinton, 1939, p. 16]. Mixtures with such minimum concentrations of pectin seemed to offer a desirable basis for these experiments, having a twofold advantage. On the one hand they can be regarded as jellies with a zero jelly strength, and on the other hand they are still solutions of pectin and so are subject to the dissociation behaviour already established.

For convenience of experiment these mixtures were prepared by fixing the composition as regards the concentrations of pectin and total solids, and adjusting the pH by trial in a series of mixtures by means of buffer solutions, so as to cover a range which would include the pH at which jellying just failed. Details were as follows.

Buffer solutions were prepared having pH values at intervals of 0.04 by partial neutralization of a lactic acid solution with KOH. (Lactic acid was used in preference to citric or malic acids because of the greater solubility of the Ca salt, required in experiments to be reported later.) A quantity of a mixed syrup of sucrose and invert sugar at 100–105° was mixed with a suitable amount of pectin paste momentarily brought to a temperature of about 95°, and the mixture was quickly poured into a series of boiling-tubes containing the required amounts of a sequence of the buffer solutions below and above the expected highest pH for setting. The pectin-sugar mixture was added up to the level of a mark on the tubes, and the latter were at once stoppered and the contents mixed. The tubes were placed in a refrigerator at about 5° and kept there for a week. Then, by inspection, those mixtures immediately above and below the jellying point were picked out, their pH determined and an estimate made of the probable exact value of the highest pH for jellying. (The pH values were determined on solutions of the mixture diluted with an equal weight of water, for greater sensitivity in the readings. By comparison with several of the less viscous undiluted mixtures, it was found that a deduction of 0.08 from the pH of the 50 % solution gave the value of the undiluted mixture.) The concentration of total solids was checked by a refractometer reading.

The amounts of the buffer solutions used were such that the total (free and combined) lactic acid in the mixtures was 0.6 %. The invert sugar amounted to about 30 % of the mixture. The results for two series of mixtures containing de-ashed red currant and raspberry pectins are given in Table 6.

Although these results are presented in the form of the upper limit of pH for jellying at certain concentrations of total solids and of pectin, they may equally well be regarded as giving the minimum pectin concentration for jellying at the respective pH values and total solids concentrations. From this viewpoint the mixtures represent a solubility or saturation limit beyond which insoluble pectin substance would separate as jelly. As they stand, these limits of solubility clearly vary considerably with pH for a given concentration of total solids. They must, however, be taken as being *total* solubilities at the respective pH values; that is, they include those pectin molecules containing dissociated acid groups (i.e. ionized pectin), as well as those molecules containing no dissociated groups. But according to the ideas developed in the

Table 6. *Upper limit of pH for jelling. (Mixtures buffered with potassium lactate-lactic acid)*

Analytical details of pectins:						
As NaOH % of calcium pectate yield						
	Free acidity	Saponification value	Alkalinity of ash	Equivalent weight		
Red currant No. 4	4.47	13.38	0.28	842		
Raspberry	4.28	13.23	0.34	865		
Total solids concentration				Pectin concentration		pH at which jelling just failed
No.	% by weight	g. per 100 g. water	% by weight	g. per 100 g. water	g. equiv. per 1000 g. water	
Red currant:						
1	65.8	192	0.40	1.17	0.0139	3.62
2	65.8	192	0.60	1.76	0.0208	3.75
3	69.7	230	0.20	0.66	0.0078	3.57
4	69.7	230	0.30	0.99	0.0118	3.71
5	69.7	230	0.40	1.32	0.0157	3.80
6	69.7	230	0.50	1.65	0.0196	3.86
7	72.8	268	0.12	0.44	0.0052	3.76
Raspberry:						
1	64.0	178	0.50	1.39	0.0160	3.57
2	67.0	203	0.35	1.06	0.0123	3.62
3	67.0	203	0.50	1.51	0.0175	3.74
4	70.0	233	0.20	0.67	0.0077	3.64
5	70.0	233	0.35	1.17	0.0135	3.73
6	70.0	233	0.50	1.67	0.0193	3.82
7	72.6	265	0.20	0.73	0.0084	3.79
8	72.6	265	0.35	1.28	0.0148	3.88

introduction it is only the latter which are capable of entering into jelly formation, and hence it is the *partial* solubility (in the sense used by Michaelis [1926]) of this non-ionized pectin which must be regarded as reaching a solubility limit in each of these mixtures.

At this point it is necessary to take account of a fundamental peculiarity of the pectin molecule, viz. the possession of a variable degree of acidity or equivalent weight. This arises, of course, from the varying degree of esterification it may have. For the "effective" ionization of a molecule of given chain

Fig. 2. Diagrammatic representation of critical degree of dissociation of the acid groups in the molecular chain of non-jellying molecules. (Esterified carboxylic groups not indicated.)

length to prevent it from participating in jelly formation, it would seem *prima facie* necessary and sufficient that there should be a particular spacing of ionized groups (dissociated acid groups) along the molecular chain (Fig. 2 (i)). Clearly

this spacing can only coincide with the spacing of the acid groups for one particular degree of esterification of the molecule (as at (i)).

At any lower degree of esterification, the acid groups in the chain will be more numerous and hence more closely spaced, and it will be unnecessary for every group to be dissociated to inhibit jellying. This condition is seen diagrammatically in Fig. 2 (ii); the three ionized groups assumed to be necessary for inhibition of jellying are still shown, but there are now two other groups exposed by de-esterification, and these need not necessarily be dissociated. In a molecule which is effectively ionized and therefore incapable of jellying, the ratio of dissociated to total (non-esterified) acid groups represents a critical minimum degree of dissociation within that molecule for its particular spacing of acid groups. This critical degree of dissociation in the ionized molecule will be called C ; it is the ratio of dissociated to total unesterified acid groups in the effectively ionized molecule.

An alternative interpretation is to assume that all the acid groups of the molecule are dissociated, but that association occurs to a certain degree between these ionic groups and the K ions; C is then a factor which takes account of the extent of this association (which would be expected to vary with the spacing of the groups in the chain).

C may have values from 1 downwards, according to the degree of esterification of the molecule. In the mixture of variously esterified molecules constituting an ordinary pectin, C is unlikely to reach the value 1 for all molecules. The only way in which this critical proportion can be dealt with in a practical sense is as a statistical average of its separate values for the individual molecules, in the way in which other properties have been dealt with. In general, therefore, the average value of C for a pectin may be expected to be less than 1.

The data of Table 6 can now be examined with a view to evaluating C for particular cases.

Let $[P]$ be the concentration of total pectin in one of the mixtures (in g. equivalents per 1000 g. water). Suppose in the first instance that in the effectively ionized molecules (i.e. those incapable of jellying) all the acid groups are dissociated ($C=1$), and the equivalent concentration of this ionized pectin is $[P^-]$. Then we have

$$K = \frac{[H^+][P^-]}{[P] - [P^-]},$$

whence

$$[P^-] = \frac{K[P]}{K + [H^+]}$$

But according to the hypothesis put forward, in general only a fraction C of the acid groups in the effectively ionized pectin molecules is dissociated. Hence the actual concentration of the latter is

$$\frac{[P^-]}{C} = \frac{K[P]}{C(K + [H^+])} \quad \text{.....(5)}$$

This may now be converted for convenience into the form g. per 100 g. water. If p is the concentration of total pectin expressed in this form, then that of the effectively ionized pectin (expression (5)) becomes

$$\frac{K[P]}{C(K + [H^+])} \times \frac{p}{[P]} \text{ g. per 100 g. water,}$$

which simplifies to

$$\frac{Kp}{C(K + [H^+])} \quad \text{.....(6)}$$

We can return now to the consideration of the non-ionized pectin, i.e. that which is capable of jellying but which in these mixtures does not in fact do so

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**Note that figures for pectin concentrates on the diagram should be
in reverse order as in legend**

because it is present only at its solubility limit. Its concentration, s , is clearly the difference between the total and the ionized pectin, viz.

$$s = p - \frac{Kp}{C(K+[H+])} \text{ g. per 100 g. water.} \quad \dots\dots(7)$$

The values of K applicable can be read off with sufficient exactness from Fig. 3, which has been plotted from the data for the red currant pectin of Table 1 to facilitate selection of K values appropriate to the pH and pectin concentration

Fig. 3. Variation of dissociation "constant" with pH at several concentrations of pectin. (Red currant pectin No. 1.)

0.26 g. pectin per 100 g. water	x
0.51 g. pectin per 100 g. water	o
0.94 g. pectin per 100 g. water	+
1.34 g. pectin per 100 g. water	Δ

of the mixtures. Taking the first two mixtures of Table 6, the K values are 3.0×10^{-4} and 2.8×10^{-4} respectively. Hence from (7), the concentrations or solubilities of non-ionized pectin are

$$1.17 - \frac{0.65}{C} \text{ and } 1.76 - \frac{1.08}{C}.$$

The total solids concentrations in these two mixtures were identical, as also were the temperatures, and it seems a not unreasonable assumption that the solubility of this non-ionized pectin is the same in both cases.

Equating the two expressions, therefore,

$$1.17 - \frac{0.65}{C} = 1.76 - \frac{1.08}{C}$$

whence $C = 0.73$.

The same method of evaluating C may now, by taking them in pairs, be applied to the four red currant pectin mixtures Nos. 3-6 of Table 6, which each had the same concentration of total solids, and to the raspberry mixtures Nos. 2-8. The data and results of these calculations are shown in Table 7.

The differences between the several values of C obtained for each pectin are not significant in view of the limits of accuracy of the observations and quantities involved. Hence it is permissible to take the averages as being more correct than the individual results. These averages are shown in the table, and it appears that they are practically identical for the two pectins.

It is now possible to substitute the average value of C in the formula (7) for the concentration of non-ionized pectin. This has been done for each mixture,

and the results are shown in the last column of Table 7. These concentrations are the solubilities of the non-ionized pectin in mixtures with total solids concentrations as shown in Table 6, and at a temperature of approximately 5°.

Table 7. *Critical dissociation of pectins and solubilities of the non-ionized pectin*

No.	$[H^+] \times 10^{-4}$ (at upper limit for jellying)	K (from Fig. 7) $\times 10^{-4}$	Concentration of ionized pectin (from (6)) g. per 100 g. water	C (by equating values for (7))	Solubility of non-ionized pectin g. per 100 g. water
Red currant:					
1	2.40	3.0	$\frac{0.65}{C}$	0.73	0.28
2	1.78	2.8	$\frac{1.08}{C}$		0.28
3	2.69	2.9	$\frac{0.34}{C}$	0.70	0.19
4	1.95	2.7	$\frac{0.57}{C}$		0.21
5	1.58	2.6	$\frac{0.82}{C}$	0.76	0.20
6	1.38	2.5	$\frac{1.06}{C}$		0.20
7	1.74	2.3	$\frac{0.25}{C}$	0.73	0.10
			Average	0.73	
Raspberry:					
1	2.69	3.1	$\frac{0.74}{C}$	0.73	0.36
2	2.40	3.0	$\frac{0.59}{C}$		0.24
3	1.82	2.8	$\frac{0.92}{C}$		0.23
4	2.29	2.8	$\frac{0.37}{C}$	0.67	0.16
5	1.86	2.8	$\frac{0.70}{C}$		0.20
6	1.51	2.7	$\frac{1.07}{C}$	0.74	0.18
7	1.62	2.5	$\frac{0.44}{C}$		0.12
8	1.32	2.5	$\frac{0.84}{C}$	0.73	0.11
			Average	0.72	

It is evident that the partial solubility of the pectin decreases as the total solids concentration in the jellying mixture increases. The average values for the partial solubility at each total solids concentration are shown in Table 8, and are plotted against the total solids concentrations in Fig. 4.

The results for the two pectins fall substantially on the same curve.

Table 8. *Solubility of non-ionized pectin at varying total solids concentration*

Temperature approximately 5°		
	Total solids g. per 100 g. water	Solubility of non-ionized pectin g. per 100 g. water
Red currant pectin	192	0.28
	230	0.20
	268	0.10
Raspberry pectin	178	0.36
	203	0.24
	233	0.18
	265	0.12

Fig. 4. Solubility of non-ionized pectin: variation with total solids concentration.

With potassium buffer } Red currant pectin 4 x With sodium buffers } Raspberry pectin +
 } Raspberry pectin o

Comparison of effects of K and Na buffers

Jellies prepared with Na buffers are decidedly inferior in strength to similar jellies prepared with K buffers. In comparisons carried out for other purposes, jellies of a given rigidity could be obtained with K buffers at a pH about 0.2 higher than with Na buffers. It has already been seen that the dissociation "constant" of the pectin is the same when either of these metals is used as neutralizing agent.

Similar experiments to those already described were carried out to find the upper limit of pH for jellying in mixtures with Na lactate buffers. The raspberry pectin of Table 6 was used, and the data and results are shown in Table 9.

The value of *C* was found to be appreciably lower than in the K series. This means that a smaller proportion of the acid groups is dissociated for effective ionization of the molecule; in other words, the spacing of ionized groups in the molecular chain is wider in the Na series. This may be taken as evidence of a specific ionic association effect.

Table 9. *Critical dissociation of pectin and solubility of non-ionized pectin in raspberry pectin jellies with Na buffers*

No.	Total solids concentration		Pectin concentration			pH at which jellying just failed	[H ⁺] × 10 ⁻⁴	K (from Fig. 7) × 10 ⁻⁴	Conc. of ionized pectin g. per 100 g. water (from (6))	Calculated value of C	Solubility of non-ionized pectin g. per 100 g. water
	% by weight	g. per 100 g. water	% by weight	g. per 100 g. water	g. equiv. per 1000 g. water						
1	64.0	178	0.50	1.39	0.0160	3.37	4.27	3.5	$\frac{0.63}{C}$		0.42
2	67.0	203	0.35	1.06	0.0123	3.42	3.80	3.4	$\frac{0.50}{C}$	0.64	0.29
3	67.0	203	0.50	1.52	0.0175	3.52	3.02	3.3	$\frac{0.79}{C}$		0.29
4	70.0	233	0.35	1.17	0.0135	3.53	2.95	3.2	$\frac{0.61}{C}$	0.66	0.23
5	70.0	233	0.50	1.67	0.0193	3.62	2.40	3.1	$\frac{0.94}{C}$		0.22
6	72.6	265	0.20	0.73	0.0084	3.57	2.69	2.9	$\frac{0.38}{C}$	0.66	0.15
7	72.6	265	0.35	1.28	0.0148	3.68	2.09	2.9	$\frac{0.74}{C}$		0.14
Average										0.65	

The partial solubilities were rather higher than in the K series (v. Fig. 4), though the differences are possibly not greater than might be due to experimental error.

Alternative method for finding the solubility of the non-ionized pectin

A more direct method for finding the solubility of the non-ionized pectin consists in finding the limit of pectin concentration for jellying at a pH low enough for the dissociation of the pectin to be substantially suppressed.

Two mixtures of low pectin concentration were prepared from the raspberry pectin of Table 6, having in place of a buffer solution a mixture of lactic acid (the same proportion as in the earlier mixtures) and HCl to give a pH near 2. As this pH is approached [H⁺] becomes large in proportion to K, and the concentration of non-ionized pectin approaches the total pectin concentration (cf. formula (7)).

The procedure in preparing the mixtures was as before, and the data for their composition and condition as regards jellying, after a week in the refrigerator at approx. 5°, are shown in Table 10.

Table 10. *Jellying behaviour at low pH*

No.	Total solids concentration		Pectin concentration			pH (50% solution)	Appearance
	% by weight	g. per 100 g. water	% by weight	g. per 100 g. water	g. equiv. per 1000 g. water		
1	70.1	234	0.08	0.27	0.0034	2.25	Very weak jelly
2	70.6	240	0.05	0.17	0.0021	2.25	Syrup with only traces of jellying

It is evident that the pectin concentration of No. 2 was very close to the limiting value for jellying. Hence, in view of the suppression of ionization, the solubility of the non-ionized pectin can be but very little less than 0.17 g. per 100 g. water, even if a wide latitude for the values of K and C is allowed in applying formula (7). From Fig. 4 the partial solubility for a total solids concentration of 240 should be approximately 0.16, and with this figure the indications of the present experiment are in substantial agreement.

This method of finding the partial solubility of a pectin offers a means of avoiding certain complications such as the effect of the particular composition of the juice or buffer mixture on the ionization behaviour. This may make it unnecessary to separate the pectin in the ash-free condition in order to investigate its fundamental jellying properties.

Effect of partial de-esterification

Since de-esterification results in an increase in the proportion of acid groups in the molecule, and hence in the spacing of these groups in the molecular chain, it may be expected to give rise to changes in the critical degree of dissociation within the molecule, and hence in the jellying behaviour.

It has been shown above (Table 4) that de-esterification appears not to cause any appreciable change in the dissociation "constant" for a given degree of neutralization or pH . Evidence of a change in C was however found in the case of a red currant pectin prepared from crushed fruit, in which enzymic de-esterification had occurred. The equivalent weight of this pectin was 480. The upper limit of pH for jellying was determined as before with K lactate buffers. The amount of pectin available did not permit of determinations on more than a few mixtures; the results of these, with the calculated value of C and of the partial solubilities, are shown in Table 11.

Table 11. *Upper limit of pH for jellying of partly de-esterified pectin*

Red currant pectin No. 5

Analytical details of pectin		<div> <div>Free acidity 7.82% (as NaOH % of calcium pectate yield)</div> <div>Saponification value 10.69% (as NaOH % of calcium pectate yield)</div> <div>Alkalinity of ash 0.51% (as NaOH % of calcium pectate yield)</div> <div>Equivalent weight 480</div> </div>									
		Pectin concentration					pH at which jellying just failed	[H ⁺] × 10 ⁻⁴	K (from Fig. 7) × 10 ⁻⁴	Conc. of ionized pectin (from (6)) g. per 100 g. water	Calcu- lated value of <i>C</i>
Total solids concentration		% by weight	g. per 100 g. water	g. equiv. per 100 g. water							
No.	% by weight				g. per 100 g. water	% by weight	g. per 100 g. water				
1	66.0	194	0.20	0.59	0.0123	3.20	6.31	3.7	$\frac{0.22}{C}$	0.54	0.18
2	66.0	194	0.40	1.18	0.0246	3.38	4.17	3.5	$\frac{0.54}{C}$		0.18
3	70.0	233	0.20	0.67	0.0140	3.51	3.09	3.0	$\frac{0.33}{C}$		0.06

The value found for C was appreciably lower than with the pectins of higher equivalent weight (cf. Table 7), though the reduction was not in proportion to the lowering of the equivalent weight. This may perhaps be taken to show a greater tendency to jellying in the molecules of the de-esterified pectin, since a

narrower spacing of dissociated acid groups in the molecular chain is required to prevent it.

The solubility of the non-ionized pectin was also lower than in the more fully esterified pectins (cf. Table 8).

THE JELLYING PROCESS IN ITS QUANTITATIVE ASPECTS

In the case of a jelly of pure pectin and sugar, with no buffer present but with the pectin partly neutralized by alkali, the following constituents are involved in the electrolytic equilibria in the solution before jellying commences.

- (i) Non-ionized pectin molecules (containing only undissociated acid groups).
- (ii) Ionized molecules having a critical proportion C of their acid groups dissociated.
- (iii) H ions.
- (iv) Alkali metal ions.
- (v) OH ions (in negligibly small concentration).

If the concentration of (i) is greater than the partial solubility at the particular total solids concentration and temperature of the mixture, the non-ionized pectin in excess will begin to separate and form jelly. Thereby the mass equilibrium will be disturbed, and adjustment must take place by union of H ions with anionic groups of the pectin (ii). This reinforces the concentration of non-ionized pectin (making more of it available for jellying), reduces the amount of ionized pectin and results in a rise of pH .

The separation of the excess of non-ionized pectin, and the accompanying reduction in the amount of ionized pectin and rise of pH , must continue until the concentration of the former has fallen to the level of its solubility. At this point, mass equilibrium still requires a definite relation between the concentrations of ionized and non-ionized pectin and of H ion; hence the solubility of the non-ionized pectin, in conjunction with the requirement for electrical neutrality, controls the final amount of pectin remaining in solution (i.e. unjellied) and also the final pH .

Thus, if s is the solubility of the non-ionized pectin, and p the total unjellied pectin (both as g. per 100 g. water), then from (7) above:

$$p = \frac{s}{1 - \frac{C(K + [H^+])}{K}} \quad \text{.....(8)}$$

Also the condition of electrical neutrality requires:

$$\begin{aligned} [H^+] + [Na^+] &= [P^-] \\ &= \frac{K[P]}{K + [H^+]}, \end{aligned} \quad \text{.....(9)}$$

where $[P]$ is now the concentration of unjellied pectin in g. equivs. per 1000 g. water (i.e. $[P] \times \frac{\text{equiv. wt.}}{10} = p$). From these equations both p and $[H^+]$ can be evaluated.

The rise of pH in unbuffered pectin jellies during the process of jellying can be demonstrated if the pH is determined, not on a solution of the jelly, but on the jelly itself.

In a jelly containing a buffer mixture, the case is similar except that, provided that the amount of buffer is large compared with the pectin, the pH cannot rise appreciably. Hence the value of $[H^+]$ is practically the same as the initial value (i.e. before jellying began); and since it is thus known with sufficient exactness, the evaluation of p is simplified, formula (8) alone being necessary.

In both the unbuffered and the strongly buffered jellies the non-ionized part of the finally unjellied pectin is the same in amount, being equal to the partial solubility of the pectin. The amount of the ionized part however is different in the two cases, since it is in equilibrium with different H ion concentrations. In the buffered jelly it is lower than in the unbuffered (other conditions being the same initially); consequently the excess of pectin which separates as jelly is greater, and the buffered jelly is stronger. This offers an explanation of a fact observed before the present work was begun, viz. that jellies prepared from purified pectin and depectinized fruit juice or a buffer solution were considerably stronger than jellies prepared from the purified pectin only, at the same initial pH.

Considering now the amount of pectin which separates as jelly, this is given by

$$p_t - p = \frac{s}{1 - \frac{K}{C(K + [H^+])}}, \quad \dots\dots(10)$$

where p_t is the concentration (as g. per 100 g. water) of total pectin in the jelling mixture.

Values for p_t , s , C and $[H^+]$ can be inserted in this formula from the known data. The value of K , however, should be that corresponding to the concentration not of the total pectin, but of the unjellied part, p , which is not known until K itself has been fixed. The values of K differ so little with concentration, however (v. Fig. 3), that it is possible to choose an approximate value of K , evaluate an approximate figure for p , and so arrive at a more precise value of K for use in formula (10).

For the purpose of examining the quantitative aspects of the strength of jellies on the basis established above, data for a number of jellies prepared from the pectins of Table 6 may be considered. These jellies were prepared with K lactate or Na lactate buffers having pH values below the upper limit for setting. The general method of preparation was similar to that used in preparing the earlier mixtures, except that the sugar syrup was added to a mixture of the buffer solution and pectin paste, and the whole poured into jelly-testing boxes. The jellies were tested after remaining overnight in a refrigerator (approx. 5°), the apparatus described by Campbell [1938] being used.

The compositions and strengths of the jellies are shown in Table 12, and in addition the amount of unjellied pectin as calculated by formula (8) and of jellied pectin by formula (10). The values of K shown are the more precise values arrived at after making a first approximation, as already described.

The pectin concentrations in these jellies are expressed on the basis of a constant weight of water (100 g.). Since it is now sought to compare pectin concentrations with jelly strengths, the latter should also be expressed on this basis. The jelly strength, however, expressed in terms of rigidity (g. weight per sq. cm.), has reference to unit area of jelly [Campbell, 1938], and the two modes of expression have to be reconciled in the following manner.

Suppose two jellies have the total solids concentrations x and y g. per 100 g. water. Then the weights of each containing 100 g. water are $100 + x$ and $100 + y$ g., and their volumes are $\frac{100+x}{d_1}$, and $\frac{100+y}{d_2}$ ml., d_1 and d_2 being the respective densities. The rigidities, were they to be compared on the basis of a constant weight of water, would therefore have to be expressed on areas equivalent to these volumes, or $\left(\frac{100+x}{d_1}\right)^{\frac{2}{3}}$ sq. cm. and $\left(\frac{100+y}{d_2}\right)^{\frac{2}{3}}$ sq. cm. re-

spectively. The rigidity of the second jelly would thus have to be expressed on an area $\left[\frac{(100+y)d_1}{(100+x)d_2}\right]^{\frac{2}{3}}$ times that on which the rigidity of the first was expressed. Hence, if the rigidity of the first is determined on the usual basis as g. per sq. cm., that of the second, as determined on the same basis, would have to be multiplied by $\left[\frac{(100+y)d_1}{(100+x)d_2}\right]^{\frac{2}{3}}$ for the purpose of comparison with the pectin concentration.

For present purposes, the densities of the jellies may be taken as equal, $d_1 = d_2$. Also, if x is 233, as in Nos. 3-8 of Table 12, the multiplying factor for other jellies becomes

$$\left[\frac{100+y}{333}\right]^{\frac{2}{3}}$$

For $y = 192$, the factor is 0.92 and for $y = 268$, it is 1.07.

These corrections have been applied to the rigidities of Table 12 where necessary.

Table 12. *Comparison of amount of jellying portion of pectin with strength of jelly obtained*

No.	g. per 100 g. water		pH of jelly	[H] $\times 10^{-4}$	K $\times 10^{-4}$	g. per 100 g. water			Jelly strength (corrected) (units of rigidity)	Ratio: Jelly strength Jellied pectin
	Total solids	Pectin p_t				s Tables 8 and 9	(from Unjellied pectin (from (8))	(p) (from (10))		
Red currant (K buffers)										
1	192	1.18	3.14	7.24	3.8	0.28	0.53	0.65	15.6	24
2	192	1.18	3.30	5.01	3.5	0.28	0.64	0.54	11.5	21
3	233	0.67	3.10	7.94	3.7	0.19	0.34	0.33	7.0	21
4	233	0.67	3.25	5.62	3.4	0.19	0.40	0.27	6.0	22
5	233	0.67	3.40	3.98	3.2	0.19	0.49	0.18	3.3	18
6	233	1.33	3.19	6.46	3.5	0.19	0.37	0.96	20.5	21
7	233	1.33	3.39	4.07	3.1	0.19	0.47	0.86	19.2	22
8	233	1.33	3.54	2.88	2.9	0.19	0.61	0.72	13.0	18
9	268	0.73	3.22	6.03	3.0	0.10	0.18	0.55	12.3	22
10	268	0.73	3.40	3.98	2.7	0.10	0.22	0.51	11.1	22
11	268	0.73	3.50	3.16	2.6	0.10	0.26	0.47	8.8	19
Raspberry (K buffers)										
1	233	0.67	3.05	8.91	3.7	0.18	0.30	0.37	4.2	11
2	233	0.67	3.29	5.13	3.3	0.18	0.39	0.28	2.0	7
3	233	1.33	3.10	7.94	3.6	0.18	0.32	1.01	13.8	14
4	233	1.33	3.36	4.36	3.2	0.18	0.44	0.89	10.2	11
Raspberry (Na buffers)										
1	233	1.33	3.28	5.25	3.5	0.22	0.57	0.76	3.3	4
2	233	1.33	3.10	7.94	3.7	0.22	0.43	0.90	5.8	6

The results appear to give some indication of a direct quantitative basis for the jelly strength. In the final column of the table the ratio of the jelly strength to the concentration of jellied pectin is shown; for each pectin and type of buffer salt there is some approach to a constant ratio. Although the jelly strengths would presumably have increased somewhat on prolonged storage, probably the approach to constancy of this ratio would not be materially affected. Indeed, there is some possibility that it would be improved, as a tendency will be noted for the recorded ratio to be low where the pH is rather high. Morris [1935] has shown that the rate of setting of pectin jellies may be delayed by raising the pH by addition of a buffer salt. In the present series, the concentration of buffer

salt is obviously greater where the pH is higher, and hence a greater relative rise in jelly strength, and therefore in the ratio, might be expected on longer standing in those cases where the latter is at present low.

Accepting the constancy of this ratio, therefore, it may be taken to mean that when allowance has been made for the solubility of the non-ionized pectin and for the ionized pectin in equilibrium with it, the rest of the pectin builds up a jelly structure whose strength is directly proportional to the amount of pectin composing it.

The ratio for the raspberry pectin (with K buffers) is only about half that for the red currant pectin. This would appear to indicate an intrinsic difference between the jellying powers, not merely of the entire pectins, but of the portions actively engaged in jelly formation. Such an intrinsic difference is clearly independent of the arbitrary conditions of pH , total solids concentration and pectin concentration, and is likely to be related to some fundamental property such as the degree of polymerization of the molecule.

Some consequences pH-jelly strength relations

As an outcome of the present work four characteristics of pectins have been found which are effective in regulating the strengths of jellies under particular conditions:

(1) Dissociation of the acid groups in accordance with an apparently variable dissociation "constant".

(2) A critical degree of dissociation of the acid groups of the individual molecule for effective inhibition of the jellying propensity.

(3) The solubility of the non-ionized pectin.

(4) The intrinsic jellying capacity of the non-ionized pectin present in excess of the solubility limit.

An examination of the possible variations and combinations of these factors shows that they can account to a considerable degree for the main features of the jellying behaviour of pectins under various conditions.

The general relation between pH and jelly strength, for example, when premature setting is avoided, is expressed by a maximum jelly strength in the neighbourhood of pH 2 or somewhat below, with the strength declining as the pH is raised, eventually more or less sharply as the upper limit of pH for jellying is approached [Tarr, 1926; Lüers & Lochmüller, 1927; Olsen, 1934; Lampitt & Money, 1939]. This fall towards the upper limit tends to be sharp when the pectin concentration is high, and more gradual when it is lower [Stuewer *et al.* 1934]. The difference is one which can be seen to be necessitated by the interplay

of the four characteristics of the pectin. Thus, a case may be taken where

Fig. 5. Typical calculated pH-jelly strength curves at varying pectin concentration.

$C=0.73$, the partial solubility is 0.20, and the jelly strength ratio is 21, a combination of figures such as was found for the red currant pectin of Tables 6 and 12. Jelly strengths corresponding to these data can be calculated for any total pectin concentration p_t and at any pH by means of formula (10) above, the values of K appropriate to the pH being taken from Fig. 3. Fig. 5 shows the pH -jelly strength curves calculated in this way for three concentrations of pectin; it can be seen that the slope towards the upper limit of pH is much steeper for 0.30% than for 0.20% pectin. The general resemblance of these curves to those of Stuewer *et al.* [1934, Fig. 5] is clear, considering only those portions of their curves which are above the "optimum" pH . In a separate communication by Olsen [1934], the occurrence of an optimum pH was shown to be an effect due to premature gelation. Had this been avoided, it is possible that the pH -jelly strength curves of Stuewer *et al.* would have been throughout in general conformity with the requirements of the present hypothesis. (Detailed conformity cannot of course be checked in the absence of knowledge as to the particular numerical magnitudes involved.)

The fact that, when premature setting is avoided, the jelly strength exhibited at the pH of 2 is not far from the maximum attainable is another relation necessitated by the factors under discussion. It is this relation which makes it desirable to compare the jelling powers of pectins at this pH [Hinton 1939, p. 16]. Taking formula (10) above for the amount of pectin capable of separating as jelly, the limit when $[H^+]$ is very large compared with K is

$$p_t - s. \quad \text{.....(11)}$$

At a pH of 2, taking round figures of 5×10^{-4} for K and 0.7 for C , the amount capable of jelling is

$$p_t - 1.07s. \quad \text{.....(12)}$$

For jellies containing usual concentrations of pectin, the difference between (11) and (12) is hardly greater, proportionally, than the possible experimental error of the jelly strength determination. Thus, in the comparisons of jelling power discussed elsewhere [Hinton, 1939, Parts IV and V], since the concentration of pectin was 0.35% and the water content 35%, $p_t = 1.00$; and if s is assumed to be as high as 0.30, expressions (11) and (12) take the values 0.70 and 0.68 respectively. Hence the jelling powers recorded at pH 2 should be within about 3% of the theoretical maximum. The discrepancy of course would be more significant if the pectin concentration were low, or if the value of s were raised by increasing the water content.

Another direction in which jelling behaviour is interpreted is in regard to the effect of total solids concentration. The effect of lowering the latter is to shift the pH -jelly strength curve downwards to a lower pH range, the shift becoming more marked as the total solids concentration becomes lower [Stuewer *et al.* 1934]. Taking once more the case of the red currant pectin above, Fig. 6 shows the curves calculated for three concentrations of total solids, for jellies containing 0.25% pectin. The resemblance to the relevant portions of the curves of Stuewer *et al.* [1934, Fig. 3] is again evident, and in this case also the conformity of the latter with the present ideas might have been extended to the whole curves had premature gelation been avoided.

The possibility of variable values for C and s provides for an explanation of a further phenomenon to which some attention has already been given [Hinton, 1939, p. 66]. In the case of two pectins which only differ in their degrees of esterification, the more highly esterified pectin forms jellies up to a higher limit of pH than the other, yet its jellies may at a low pH be slightly weaker; there is

a crossing over of the pH-jelly strength curves. The adequacy of the factors under discussion to account for an effect of this kind can be seen if the full pH-jelly strength curves are plotted for a particular case. For this purpose the

Fig. 6. Typical calculated pH-jelly strength curves at varying total solids concentration.

Fig. 7. Typical calculated pH-jelly strength curves at different degrees of esterification of the pectin.

data found for the red currant pectins of Tables 7 and 11 may be used. Fig. 7 shows the curves for these pectins at a concentration of 0.4%, with 66% of total solids. The rigidity factor of 21 (v. Table 12) has been used in calculating the jelly strengths from the amounts of jellied pectin as given by formula (10). The curves, it can be seen, reproduce the essential features of the diagrammatic curves previously suggested. The calculation brings out the reason for the crossing over, viz. the decrease in the solubility (s) of the non-ionized pectin which accompanies the fall in the value of C resulting from the de-esterification.

Attention has already been drawn to the fact that the upper limit of pH for setting is raised when the degree of esterification is increased [Hinton, 1939, p. 65]. This rise of the pH limit can be accounted for if the critical dissociation C is assumed to become greater with increasing esterification. This assumption is a justifiable one in view of the demonstration (cf. Tables 7, 11) that C becomes smaller with partial de-esterification, as well as on the *prima facie* grounds already discussed (p. 1220).

The magnitude of this rise in the value of C can be approximately evaluated in the case of a pectin which was prepared by esterifying an orange pectin with methyl alcohol. The equivalent weights of the pectin before and after the esterification were 615 and 1480 respectively. Jellies were obtainable up to a pH of about 4.2, whereas the limit for the untreated pectin under similar conditions was 3.4 (total solids, 70%; pectin, 0.21%, $p=0.70$; K citrate buffers; temperature, approx. 10°). For the untreated pectin a value for C of 0.62 may be taken (i.e. linearly intermediate between the values for the pectins of Tables 7 and 11, with equivalents of 842 and 480). This, with $K=3.3 \times 10^{-4}$, gives the solubility (s) of the non-ionized pectin under the conditions of the test, by formula (7), as 0.19 g. per 100 g. water. (This is a not unreasonable figure, compared with a value suitably intermediate between the earlier results of Tables 8 and 11, in view of the slightly higher temperature in this case.)

If no increase in s occurred as a result of the esterification, then C can be approximately calculated for the esterified pectin by applying formula (7). Taking K as approximately 1.6×10^{-4} (by extrapolation from the data of Table 4), the formula gives

$$0.19 = 0.70 - \frac{1.6 \times 10^{-4} \times 0.70}{C(1.6 + 0.6) \times 10^{-4}},$$

whence

$$C = 1.0.$$

This means that for effective ionization of the pectin molecules when the equivalent weight reaches about 1480 all the acid groups must be dissociated.

Had s been increased by the esterification, the calculation would give C as greater than 1, which would be meaningless. It seems, therefore, that s is unlikely to have increased appreciably. On the other hand, it is unlikely to have decreased, in view of the fact that a decrease was found to accompany de-esterification.

It may be pointed out before concluding that it has been the intention in this paper to demonstrate the general principles governing the separation of pectin as jelly, rather than to claim any absolute numerical validity for the factors and quantities evaluated from the experimental work. The numerical values obtained are, in fact, probably affected to some extent by certain experimental approximations which have been made.

For example, in the calculations of the latter part of the paper the appropriate values for the dissociation "constant" have been taken from Fig. 3, which gave the values for one particular pectin. Although other pectins have been found to have substantially the same system of "constants" as this, yet minor variations cannot be ruled out altogether; and if the values for this pectin are not strictly applicable to others, the calculated values of the jelling factors will be affected. To take an illustration, if the values of K used in Table 7 are taken from the extrapolated data for the orange pectin of Table 1, the numerical value of C averages 0.82, and the results for the solubility of the non-ionized pectin are somewhat increased. These changes, however, do not affect the general argument as to the factors controlling the extent of jelly formation under given conditions.

Again, in finding the upper limit of pH for jelling, the pectin mixtures were allowed to stand for a week to develop any signs of jelling. Had they been left longer it is possible that slightly higher limits might have been found. The pH limit after one day only was observed in a number of the mixtures, and was never more than about 0.1 below the figure after a week; hence further changes of any magnitude would seem unlikely. Any further small upward shifts of the

pH limits would tend to give slightly higher values for C , and slightly lower solubilities for the non-ionized pectin. Again, however, the general nature of the results would be unaffected.

SUMMARY

The electrolytic dissociation of pectins in dilute solution was investigated. The dissociation "constant" was found to diminish with increasing degree of neutralization with alkali, from a value of about 5×10^{-4} for the free acid condition in a concentration of approx. 1 g. per 100 g. water.

Dilution caused a small decrease in the value of K at a given pH or degree of neutralization.

No significant alteration of the dissociation "constant" was caused by boiling a pectin solution for 1 hr., or by enzymic de-esterification or by admixture with citric acid.

From the results of determinations of the upper limit of pH for the jelling of pectin-sugar mixtures on the assumption that jelly formation is only participated in by molecules in the non-ionized condition, that it is shown that the concentration of such non-ionized pectin must exceed a certain solubility, or saturation limit, which varies with the total solids concentration of the mixture. It is necessary to assume that in general the pectin which is ionized and incapable of jelling has a certain proportion only of its acid groups dissociated. This proportion varies with the nature of the buffer salt present (potassium or sodium), and with the degree of esterification of the pectin. The strengths of jellies from any one pectin, with a given buffer salt, were found to be proportional to the amount of the excess of non-ionized pectin above the limit of its solubility. For different pectins, the ratios of jelly strength to amount of jelling substance differed.

It is shown that many of the phenomena of the jelling of pectins, especially in regard to pH conditions, can be accounted for by the proposed hypothesis.

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