CXIX. A METHOD FOR THE ESTIMATION OF THE SALT CONTENT FROM THE p_H VALUE OF APPLE JUICE, AND SOME COMPARATIVE ANALYSES OF THE MINERAL CONTENT OF THE JUICE AND WHOLE APPLE.

BY DOROTHY HAYNES AND JANET W. BROWN.

*From the Department of Scientific and Industrial Research and the
Department of Plant Physiology and Pathology, Imperial College
of Science and Technology, London.*

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MEASUREMENTS of the hydrogen ion content of the juice of apples were undertaken by one of the authors (D. H.) some years ago in order to ascertain whether any sudden change in the value of this property marked the beginning of breakdown. The experiments were carried out over two seasons and no sudden change of p_H occurred, although alterations in the relation of this property to the titratable acidity were observed. These results could not be completely interpreted without the aid of mineral analysis, but now that the other author (J. W. B.) has elaborated methods for a complete mineral analysis of the apple, a comparison between salt content and p_H has been made possible, and may be usefully discussed.

It is shown below that if the p_H and titratable acidity of the juice of acid apples is measured, it is possible to obtain a fairly reliable idea of its salt content by comparison with the acidity and p_H of mixtures of malic acid and potassium malate of known concentration. Since these measurements can be carried out rapidly, while mineral analyses are long and laborious, this method of obtaining an approximate estimate of salt content is likely to prove to be of value, if the soluble salt contained in the expressed juice is a measure of that contained in the whole apple. Previous examinations of successive fractions of expressed juice have been shown to give little or no evidence of any retention of sugars or acids in the press residue, for the value of p_H in these fractions has been found to correspond with the titration, which indicates that the concentration of salts does not vary sufficiently to affect the hydrogen ion concentration appreciably [Haynes and Judd, 1919; Haynes, 1921]. More delicate methods of mineral analysis have, however, brought to light small differences in the juice expressed from the apple under different conditions, which suggest that the acid sap dissolves certain constituents of the cell wall. These observa-

tions, and the relation of p_H to mineral content, are discussed below under the following headings.

(1) The hydrogen ion concentration (p_H) of mixtures of malic acid and potassium malate.

(2) Comparisons of the "equivalent potash" calculated from measurements of p_H and titratable acid in certain juices with the potash equivalent of the potash, magnesia and lime found by analysis in the same juices. The term "equivalent potash" is introduced to express the effect upon the p_H of all the cations present. It represents the amount of potash, combined as malate, which would be required to raise the p_H of a solution of malic acid, of concentration equal to that of the titratable acid present, to the observed value. In apple juice the "equivalent potash" is only slightly greater than the actual quantity of potash present, since the percentage of potash in the ash is very large compared with the percentages of lime and magnesia, the chief other cations present. The further assumption involved, that all the acid is present as malic acid, is discussed below.

(3) Titrations and p_H values of the juice of various apples at intervals during storage and their "equivalent content" of K_2O .

(4) A comparison between the mineral analysis of apples with that of their expressed juice, and the changes in the composition of the juice during a period of storage.

The methods used both for p_H determinations and mineral analyses have been already described [Haynes, 1925; Brown, 1926], but one point of difference may be noted. Measurements of p_H were made as before in a water-bath kept at a temperature of 25° ; as the whole apparatus could not be submerged a small difference of temperature usually existed between the parts in air and water; in the previous measurements¹ this was compensated by an alteration in the value of E in the factor $\frac{E-\beta}{f(t)}$; in the present instance the apparatus was so arranged that for small differences of inside and outside temperature the correction became negligible.

(1) *The hydrogen ion concentration (p_H) of mixtures of malic acid and potassium malate.*

In order to investigate the relation between the p_H and the salt content of the juice, a number of measurements of p_H were carried out in mixtures of malic acid and potassium malate of known composition. All the measurements were made in duplicate on solutions prepared from good commercial specimens of malic acid; the measurements were, however, confirmed by further observations made with solutions of recrystallised malic acid. The more concentrated acid solutions were made up and standardised by titration with phenolphthalein as indicator, the weaker solutions being obtained from these by dilution. Potassium malate solutions of concentration $0.2N$, the strongest

¹ This difference was formerly erroneously attributed to incomplete saturation.

solutions used, were prepared by mixing equal volumes of carefully standardised solutions of malic acid and potassium hydroxide of double this strength; other solutions were obtained from these by dilution. The p_H values corresponding with the final concentrations of these various mixtures are shown in Fig. 1.

Fig. 1. p_H of mixtures of malic acid and potassium malate. The figures on the curves denote concentrations of K malate. Concentrations in mg. equivalents per litre.

The range of p_H covered is from 2.2 to 5.7, *i.e.* of hydrogen ion concentration from 63×10^{-4} to 0.02×10^{-4} , which is a considerably larger range than that shown by the juice. In Fig. 1 p_H is plotted against the logarithm of the titratable acidity in order to make it evident that p_H approximates to a linear function of the acid concentration. The curves of Fig. 1 show very plainly the effect of the presence of salts upon the hydrogen ion concentration; they represent the results of the interaction of three constituents, malic acid, potassium malate and potassium hydrogen malate. In the first part of every curve for the solutions containing salt, the only acid present is potassium

hydrogen malate; the second part of the curve shows the buffer effect of this latter compound upon malic acid. It is evident that the buffer effect increases considerably as the proportion of salt increases. This is probably of some physiological importance, for there is a considerable difference between the salt content of different varieties of apples, and in the same variety grown on different soils or grafted on different stocks.

Before proceeding to a direct comparison between the salt content as estimated from measurements of p_H and titratable acidity, and that found by analysis, some further discussion of the fundamental assumptions involved is required. The presence of cations other than potassium has already been mentioned, and it has been pointed out that the joint effect of all cations may be estimated as "equivalent potash"; for in such a dilute solution as apple juice the salts of organic acids present are completely dissociated. This assumption is supported by the results of Table I, which show that where the amount of malic acid is very small, calcium malate produces very nearly the same effect as an equivalent quantity of potassium malate. This will also be true of the corresponding magnesium and other similar salts; the buffer effect of other cations can therefore be calculated with sufficient accuracy in terms of potassium.

Table I. p_H of mixtures of malic acid and calcium malate.

Concentration of calcium malate 10 mg. equivalents per litre.		
Malic acid mg. equivalents per litre	p_H	p_H of similar solution containing potassium salt
10	3.97	4.21
50	3.00	3.12
100	2.72	2.74
150	2.55	2.57
200	2.45	2.48

The presence of acids other than malic acid has still to be considered and this is highly important, for the possibility that apple juice may contain some considerable quantity of these other acids constitutes the only serious difficulty in the use of the indirect method of estimating the salt content as described above, since the effect of the sugar and other non-electrolytes which the juice contains is so small that it may safely be disregarded. Although unfortunately the difficulty of estimating mixtures of organic acids has prevented a direct analysis of the quantities of these other acids present in the juices used, some information is available upon the general question. Citric acid appears to be the only organic acid other than malic acid that is present in any considerable quantity in apples. Franzen and Helwert [1923] found about 20% in the sample of apples which they examined; whether these apples were of any definite variety is unstated. More recently Nelson [1927] has found Winesap apples to contain only a trace of citric acid, and in York Imperial apples none could be detected. Fig. 2 shows the p_H of solutions of citric acid and potassium citrate, the corresponding values of a similar mixture of malates being given for

comparison. It will be observed that while solutions of citric acid are slightly more acid than malic acid solutions of equivalent concentration, mixtures of citric acid with its salts are slightly less acid. The effect of replacing malic by citric acid

Fig. 2. p_H of mixtures of citric acid and potassium citrate. The figures on the curves denote concentrations of K citrate. Concentrations in mg. equivalents per litre. The dotted curves show the corresponding values for malate.

in apple juice must therefore be very small, especially as to mix acids is necessarily to reduce buffering and thus to increase acidity. The p_H of the mixtures of citric and malic acids and their salts must therefore approximate very closely to that of a simple mixture of malic acid and its potassium salt in equivalent concentrations.

The presence of strong mineral acids might be disregarded but for the general use of sulphate manures, as a result of which the juice may contain quantities of sulphuric acid which are not entirely negligible. Although it is obvious that the salts of mineral acids can be only very slightly affected by admixture with malic acid, a few experiments have been made which establish that within the range of concentrations dealt with in Fig. 1 the addition of potassium chloride to solutions of malic acid tends to increase acid reaction to a very small extent, while the addition of potassium sulphate tends to decrease it, though again very slightly; in the presence of potassium malate both effects tend to disappear. Hence, since the potassium or other cation combined with mineral acid is without buffer effect, it will not be estimated by the indirect method, and therefore the presence of sulphate will tend to produce low results. This is probably a principal cause of discrepancy between the "equivalent potash" as estimated by analysis and as calculated by the indirect method.

Some experiments carried out on the effect of phosphates on the p_H of mixtures of malic acid and potassium malate within the range of concentration usually found in apple juice ($0.04N$ – $0.2N$ acid, and 3.0 – 3.75 mg. equivalents of potash per litre) showed that for the higher concentrations ($0.05N$ acid and more) the effect of replacing potassium malate by an equivalent quantity of potash as phosphate (either the dihydrogen or monohydrogen phosphate) was to raise the p_H value; thus the calculated value of potash equivalent in the presence of phosphate is higher than the true potash content. The error is of the order of 5 %. In concentrations of acid of $0.05N$ and less, the phosphate has the opposite effect, and the calculated value of potash equivalent is therefore lower than the true value. When citric acid was substituted for malic acid, for the concentrations of acids used ($0.08N$, $0.1N$, and $0.13N$) the p_H was lowered by the presence of phosphate, rendering the calculated value of the potash equivalent lower than the true value. Thus when the titratable acid is of the order of $0.1N$ the effect of phosphate on the p_H of mixtures of citrates and malates and their acids will tend to be neutralised, and this will probably occur in acid apples, but in pears and sweet apples, which contain only a small percentage of total acid, the calculation of salt content from the p_H value may be seriously affected, and further investigation is necessary before the indirect method can be used in these slightly acid juices.

Another condition tending to an undervaluation of the soluble salt content is the production of soluble pectin. This when separated shows a very marked capacity for absorbing salts and may well exert a similar action in the natural medium, but the quantity of soluble pectin present is small and any removal of salts from the sphere of action brought about by its agency must be relatively unimportant.

The result of the foregoing discussion is therefore to justify the approximate estimation of equivalent potash present in apple juices from their titration and p_H values, where the titration is not too low. It must be remembered

that in this method all the cations are estimated together as "equivalent potash" so that no knowledge of the relative percentages of the different constituents is obtained, and that the result is only intended to be approximate, for it is affected by the different sources of error discussed in detail above. It is perhaps worth while to suggest that this method of analysis might be extended to non-acid biological material, by adding malic acid of known concentration to the solution of the ash, and measuring the p_H , but the method will probably be found to be most valuable if used for the preliminary examination of material upon which it is intended to make a detailed mineral analysis.

Table II. *Comparison between (a) mineral content of the whole apple and juice, (b) direct and indirect estimation of the mineral content of juice, using Lane's Prince Albert apples (East Malling Stocks), 1924.*

The figures represent percentages referred to fresh weight.												
Whole apple					Juice							
Estimation by analysis					Estimation by analysis				Indirect estimation from p_H value and titration			
Stock	K ₂ O	CaO	MgO	Total "equiv. K ₂ O"	CaO	MgO	Total (a) "equiv. K ₂ O"*	mg. equiv. per litre	p_H	Difference		
										Calc. (b) "equiv. K ₂ O"	between (a) and (b) in g.	as % of (a)
Type I	0.0983	0.0036	0.0068	0.1108	0.0050	0.0083	0.1262	249	2.72	0.116	0.010	7.9
Type II	0.0104	0.0031	0.0071	0.1323	0.0040	0.0069	0.1333	257	2.78	0.141	0.008	6.0
Type IX	0.0811	0.0032	0.0061	0.1008	0.0051	0.0087	0.1099	196	2.83	0.122	0.012	11.0

* Using the K₂O figures for the analysis of the whole apple, since as is shown below the potash content of the juice and the whole apple is the same within the limits of error.

(2) *Comparisons of the "equivalent potash" calculated from measurements of p_H and titratable acid in certain juices with the potash equivalent of the potash, magnesia and lime found by analysis in the same juices.*

These comparisons are shown in Tables II and III and in one of the sets of analyses in Table VII. The calculated values were obtained as explained above by finding that point in Fig. 1 whose co-ordinates are the logarithm of the titration, and the p_H of the juice under examination. The concentration of potash in the corresponding malate mixture gives the value of the "equivalent potash" concentration of the juice. In any given value of p_H and acidity plotted on the graph of Fig. 1, the value of the "equivalent potash" is found from the ratio of the vertical distances of the point from the curves of concentration of "equivalent potash" between which the point lies. It has been found convenient in practice to make from Fig. 1 a table showing p_H and potash values for an increase of 0.05 in the logarithm of the titrations. The table gives differences of p_H corresponding to a rise of 10 mg. equivalents of potash, and average differences of p_H corresponding to increases of 0.01 of the logarithm of titration. With such a table values of "equivalent potash" can be easily and rapidly read.

Table II shows an analysis of Lane's Prince Albert apples grown upon various stocks. The direct and indirect analyses of "equivalent potash" agree fairly satisfactorily for the apples grown on stocks I and II. The analyses of

the juice of the apples grown on stock IX show a greater difference, which may possibly be significant, since type IX is a dwarfing stock of unusual character. The observation, however, needs confirmation before any deductions can be drawn from it, but if confirmed it would suggest a possible method by which some of the physiological differences of this nature might be investigated.

Table III. *Comparison between potash content found by analysis of the whole apple and by calculation from p_H and titration values in 30 individual Bramley's Seedling apples.*

K ₂ O figures are given as percentages referred to fresh weight of apple.				
Log. titration mg. equiv. per litre	p_H	"Equiv. K ₂ O" calc. from p_H and titration	K ₂ O found by analysis	Difference
2.299	2.95	0.160	0.1175	0.042
2.286	2.99	0.168	0.1170	0.051
2.358	2.87	0.152	0.1167	0.035
2.318	2.88	0.140	0.1094	0.031
2.272	2.89	0.126	0.0969	0.029
2.230	2.89	0.117	0.0965	0.020
2.230	2.95	0.135	0.1120	0.023
2.274	2.95	0.148	0.1060	0.042
2.274	2.90	0.131	0.1074	0.024
2.236	2.95	0.135	0.1303	0.005
2.281	3.05	0.188	0.1148	0.073
2.290	2.98	0.163	0.1150	0.048
2.267	3.09	0.192	0.1216	0.070
2.161	2.92	0.105	0.1260	0.021
2.240	3.06	0.177	0.1690	0.008
2.297	2.93	0.152	0.1357	0.016
2.215	3.05	0.161	0.1215	0.039
2.223	3.01	0.151	0.1000	0.051
2.258	3.02	0.170	0.1455	0.024
2.137	3.07	0.137	0.1371	—
2.220	3.02	0.151	0.1600	0.009
2.292	2.96	0.160	0.1381	0.022
—	2.89	—	0.1155	—
2.334	3.02	0.189	0.1762	0.013
2.233	3.05	0.180	0.1353	0.045
2.267	3.02	0.174	0.1319	0.042
2.182	3.02	0.137	0.1128	0.024
2.185	3.01	0.135	0.1500	0.015
2.238	3.06	0.177	0.1629	0.014
2.182	3.13	0.174	0.1211	0.053

Mean values.

"Equivalent K ₂ O" calculated from p_H and titration	...	0.155
K ₂ O found by analysis	...	0.127
Difference*	...	0.028
CaO and MgO as equivalent K ₂ O†	...	0.0188
Total "equivalent K ₂ O" by analysis	...	0.146
Difference from calculated value	...	0.009 or 6.2 %‡

* Chiefly due to the inclusion of CaO and MgO in the indirect estimation.

† CaO and MgO were not estimated in the individual apples, but on a mixed sample of the same apples.

‡ Percentage calculated with reference to the amount found by analysis.

Table III gives the results of 30 analyses of individual apples; further details of these analyses have already been published [Brown, 1927]. Potash was estimated by analysis of the whole apple, but as is shown later the potash

contents of the freshly extracted juice and of the whole apple agree closely, probably within the limits of error. The "equivalent potash" estimated by the indirect method in the juice includes, as explained above, the "potash equivalent" of the lime and magnesia present, which were not estimated directly in the individual apples, owing to the small amount of material in each apple. For this reason the lime and magnesia percentages have been included in the comparison of means, the values being taken from the analysis of a mixed sample of 30 of the same apples. When the two sets of estimations are thus made comparable they agree very closely, the difference being little more than 6 %; moreover, the lime and magnesia percentages were obtained from the analysis of the whole apple and not of the juice and are therefore probably too low, for, as will be shown in the sequel, the mineral analyses of the juice and the whole apple are not the same. Where the "equivalent potash" by analysis is higher than that obtained by the indirect method this is probably to be ascribed to the presence of mineral acids, particularly sulphuric, as stated above (p. 952), but sulphate was not estimated in any of the analyses quoted owing to the special methods of ashing required. However, some samples of juice were analysed for sulphate in the early stages of this work, using the ordinary method of ashing which, according to Stockholm and Koch [1923], gives low results. In the juice of Bramley's Seedling apples from different localities 7-15 % of sulphate was found in the ash, and in a sample of the juice of Lane's Prince Albert apples 10 % of sulphate was found. It has already been pointed out that the effect of the addition of small amounts of sulphate to a buffer mixture of potassium malate and malic acid is to immobilise an equivalent amount of potash (or lime or magnesia) so far as p_H determinations are concerned, and therefore to cause the value of the "equivalent potash" calculated from p_H values to be lower than that found by mineral analysis of the juice. The correction to be added to the calculated value of the "equivalent potash" would therefore be of the order of 0.02 g. when the value of sulphate is of the order of 10 % of the total ash.

(3) *Titration and p_H of the juice of various apples and their "equivalent potash."*

During the course of this work a large number of determinations of p_H have been made on various kinds of apples, and the results are shown in Table IV. There were 10 apples in each sample and they were analysed shortly after picking. More recent experience has shown that larger samples would have been better, as samples of 10 show rather wide variations. However, it appears from the results that the salt content of apples does not vary greatly, but in Cox's Orange Pippin apples it is considerably higher than in other apples. These apples have also an unusual capacity for storing sugar and the two phenomena may be connected. Apart from this fact there is little in the results of Table IV to suggest that the percentage of "equivalent potash" in apples is determined by the variety.

Table IV. *Titration, p_H and equivalent potash of various apples.*

Variety	Locality, etc.	Titration (mg. equiv. per litre)	p_H	"Equiv- alent K_2O " (g. per 100 cc.)
Bramley's Seedling	Lincolnshire	192.0	2.90	0.141
	Near Cambridge	173.0	3.03	0.174
	Near Bristol	130.5	3.05	0.127
Lane's Prince Albert	Near Bristol.	1st picking	150.0	2.96
		2nd picking	115.0	3.05
		3rd picking	96.0	3.18
Cox's Orange Pippin	Near Bristol.	1st picking	81.0	3.51
		2nd picking	82.0	3.62
		3rd picking	63.5	3.64
Allington Pippin	Near Bristol	134.5	5.09	0.147
Lord Derby	Near Bristol.	Young trees	58.0	3.35
		Trees of medium age	60.0	3.48
		Old trees	60.0	3.47
		Cultivated soil	62.5	3.36
		Uncultivated soil	44.0	3.64
Dymock Red	Near Bristol	40.0	4.03	0.174
Sweet Alford	Near Bristol	17.5	4.16	—

Table V. *Titration, p_H and "equivalent potash" of apples in store.*I. *Lane's Prince Albert apples, 1921-2.*

Date	Titration (mg. equiv. per litre)	p_H	"Equiv. K_2O " (g. in 100 cc.)	Titration (mg. equiv. per litre)	p_H	"Equiv. K_2O " (g. in 100 cc.)
Sept. 26 before storage	115.0	3.05	0.118	—	—	—
Oct. 13	104.0	3.14	0.127	94.0	3.18	0.118
Nov. 8	107.5	3.08	0.113	81.0	3.27	0.122
Dec. 8	94.0	3.16	0.118	77.0	3.29	0.122
Jan. 11	72.0	3.25	0.104	52.0	3.67	0.141
Jan. 24	85.0	3.25	0.122	61.0	3.44	0.118
Feb. 9	—	—	—	56.5	3.48	0.118
Feb. 28	71.5	3.31	0.113	55.0	3.54	0.122
Mar. 14	75.0	3.33	0.122	45.5	3.68	0.127
Mar. 26	77.0	3.30	0.122	37.5	3.78	0.113
Apr. 4	92.5	3.24	0.132	45.5	3.61	0.113
Apr. 20	76.0	3.29	0.118	42.5	3.67	0.113
May 17	—	—	—	39.0	3.68	0.104
Mean K_2O	—	—	0.1191	—	—	0.1192

II. *Cox's Orange Pippin, 1921-2.*

Sept. 26 before storage	82.0	3.62	0.217	—	—	—
Oct. 13	78.0	3.60	0.198	35.5	4.05	0.156
Nov. 10	66.5	3.64	0.179	44.5	3.90	0.165
Dec. 8	59.0	3.74	0.179	43.5	4.03	0.188
Dec. 22	—	—	—	36.5	4.19	0.188
Jan. 11	53.5	3.85	0.188	39.5	4.19	0.212
Jan. 23	38.0	4.25	0.188	—	—	—
Feb. 9	38.5	4.13	0.188	—	—	—
Mar. 2	39.5	4.02	0.170	—	—	—
Mar. 14	36.5	4.14	0.179	—	—	—
Mar. 24	40.5	4.09	0.188	—	—	—
Apr. 4	33.5	4.30	0.203	—	—	—
Apr. 20	47.0	4.00	0.198	—	—	—
Mean K_2O	—	—	0.1871	—	—	0.1818

In order to find out whether any sudden change of p_H occurred during storage, measurements were made at short intervals on samples withdrawn from store (Table V). The change in p_H was found to vary approximately with the titration, but the "equivalent potash," which would be expected to show some decrease even at low temperatures, owing to loss of water from the apple, was found to remain almost constant, probably owing to the decreasing acidity of the juice, for, as is shown in the sequel, magnesium is extracted from the cell wall during the expression of the juice and the amount extracted decreases with acidity. The increasing proportion of phosphoric acid in the juice also tends to make the value of the "equivalent potash" rather low, and is almost certainly the cause of the abnormally low results obtained occasionally in the last sample of the season when acidity has fallen very low. (No striking example of this is shown in the tables but it has been observed in samples where the acidity of acid apples has become much reduced.) It thus appears that comparisons of "equivalent potash" should be made as far as possible in similar conditions of acidity. Table IV shows the order of variations of "equivalent potash" in successive samples. The comparison of the three sets of pickings of Lane's Prince Albert and Cox's Orange Pippin (Table IV) is of interest; the results afford evidence that the first picking of Lane's and the first two pickings of Cox's are definitely higher in salt content than those which follow. This may be a consequence of the intake of water from rain and atmospheric moisture after the salt supply from the root has ceased, or it may mean that the apple is drawing upon a store of salts which is becoming depleted. The salt content of the apple is probably a factor of some importance in relation to keeping properties and although little evidence can be adduced in support of this statement one observation of some interest may be quoted. A set of Lord Derby apples from young trees which were put into store succumbed so completely to physiological breakdown after a few weeks that only a few survivors were left; these survivors were, however, in good condition. In the original sample before storing, estimated on October 20th, the percentage of soluble salt calculated in terms of potash, as shown in Table IV, was only 0.134, but for the survivors, estimated on November 15th, it was found to be 0.188. It will be noticed (Table IV) that the original sample of the apples from the young trees contained very much less salt than a sample from older trees, but the survivors of the apples from the young trees contained about the same amount. Definite conclusions cannot of course be drawn from a single series of observations, but the facts are highly suggestive, and it may well be that the bad keeping properties of apples from young trees, which is generally recognised, is due to an intake of water in excess of that of salts.

- (4) *A comparison of the mineral content of whole apples with that of their juice and the changes in the composition of the juice during a period of storage.*

In order to reduce as far as possible the errors due to individual variation, samples composed of the different halves of the same apples were compared. Table VI, which gives the results of a preliminary investigation in which the properties of the juice from the two halves of a number of Bramley's Seedling apples were compared, shows that the mean differences between samples of the two halves of apples is very small, especially if the apples are cut longitudinally.

Table VI. *Density, p_H and acidity of the two halves of Bramley's Seedling apples.*

Apples cut longitudinally.							
Apple	Density of juice		p_H		Acidity (mg. equiv. per litre)		
1	1.051	1.051	3.11	3.15	108.5	107.0	
2	1.045	1.045	3.20	3.21	104.5	97.5	
3	—	—	3.50	3.52	73.0	67.0	
4	1.053	1.051	3.15	3.13	127.0	121.0	
5	1.050	1.050	3.40	3.30	109.0	115.5	
6	1.048	1.049	3.30	3.23	95.5	97.0	
7	1.053	1.055	3.15	3.11	125.0	129.0	
8	1.053	1.054	3.21	3.21	138.0	140.0	
Mean values	1.050	1.051	3.25	3.23	110.0	109.4	
Mean difference between two halves	0.001		0.04		0.43		
Mean deviation from mean	0.0025	0.0023	0.11	0.09	1.4	1.7	

Apples cut transversely: (a) = stalk end; (b) = calyx end.							
	(a)	(b)	(a)	(b)	(a)	(b)	
1	1.049	1.051	—	3.38	112.0	113.0	
2	1.047	1.051	3.22	3.26	100.0	112.0	
3	1.052	1.053	3.74	3.44	82.0	101.0	
4	1.046	1.050	3.21	3.15	110.5	120.0	
5	1.045	1.045	3.21	3.33	108.5	98.0	
6	1.048	1.052	3.24	3.10	117.0	128.0	
7	1.050	1.052	3.18	3.25	94.0	90.0	
8	1.048	1.049	3.18	3.16	100.5	100.5	
9	1.046	1.050	3.25	3.09	93.0	97.5	
Mean values	1.048	1.050	3.28	3.27	102.0	106.6	
Mean difference between two halves	0.003		0.11		0.89		
Mean deviation from mean	0.0017	0.0017	0.12	0.11	0.9	1.0	

Thirty Bramley's Seedling apples were used for the experiment described below. They had been in cold store at 1°, from the time of gathering until February 1st, when the experiment was begun. The apples were weighed in bulk and cut in halves, longitudinally as symmetrically as possible. One half of each apple was used at once for analysis, while the other half was stored at room temperature. The halves stored were kept on clock glasses with the cut surface immersed in paraffin wax. These were analysed in two batches of 15 apples each, one after a month and the other after 6 weeks' storage.

Precautions¹ to avoid fungal infection were taken during the cutting of the apple. Each apple was washed with a wad of cotton wool dipped in alcohol, and the cutting knife of stainless steel was sterilised by dipping in alcohol and burning before the cutting of each apple. The half of the apple to be stored, with the cut half held always downwards, was placed immediately upon a clock glass containing clean melted paraffin and previously sterilised by pouring on to it a little alcohol which was then set on fire. The stored half-apples thus treated kept as well as whole uncut apples. Out of the 30 only two suffered badly from fungal attack; both of these were in the first (random) sample analysed after 1 month's storage. The three lots of half-apples, 30 half-apples at the beginning of the experiment, 15 of the other halves after 1 month's storage and those remaining after another fortnight's storage, were all treated exactly similarly. The apples were peeled, cored, cut up and thoroughly mixed and any "bad" portion was removed. About 200 g. were weighed into platinum dishes for the estimation of total ash, potash, phosphate and magnesium in the fresh apple. The remainder was frozen and the juice expressed in the ordinary way except that boiling-tubes, covered on the outside with tinfoil, and a porcelain press were used in the place of metal vessels in order to avoid metallic contamination. In addition to the above-mentioned comparisons the juice of each of the two lots of 15 half-apples was expressed in two fractions in order to test whether the mineral matter of the runnings varied, but the second running gave very little juice, and this was not sufficient for a complete analysis. The acidity, density, and sugar content of the juice was determined immediately, and portions of the juice were measured out into platinum dishes for the estimation of total ash, potash, phosphate and magnesia. The remaining juice was heated to 70° to destroy pectinase and other enzymes, and bottled with thymol as preservative, so that the p_H of the juice of all the samples could be determined. The samples of juice and apple for mineral analysis were incinerated slowly in an electric muffle furnace, which was gradually allowed to reach a temperature of about 400°. The details of analysis were those previously described by one of the authors [Brown, 1926].

One sample of Dabinett apples was also analysed; this is a bitter-sweet variety grown for cider. The total ash, potash, phosphate and magnesia were estimated on the juice and the whole apple as before. For these apples the "equivalent potash" in the juice calculated from the p_H and titration values was very much lower (about 35 %) than that actually found by analysis. However, since the juice coagulated on heating, owing to its low acidity, before the destruction of pectinase, the p_H values were probably not dependable, and therefore the p_H and calculated "equivalent potash" have been omitted from Table VII; it may also be possible that in these apples with peculiar properties and low acidity the malate comparison is invalid.

¹ The authors desire to express their acknowledgement to Dr A. S. Horne for advice as to these precautions.

Sugar was determined on the samples of juice using Lane and Eynon's [1923] methylene blue method [Evans, 1928], and the acid by titration to a standard colour with phenolphthalein; the percentages were found from the weights in 100 cc. of juice by multiplying by the factor $\frac{100-z}{100\sigma}$, where z is the residue left from alcoholic extraction (taken as cell wall material) and σ is the specific gravity of the juice.

The results of these analyses in terms of comparable quantities are shown in Table VII. It is at once clear that the ash content of the juice is markedly higher than that of the whole apple. This is not due to any deficiency of ash in the cell wall, for this, which is normally less than 2 % of the whole, has been found after exhaustive extraction with alcohol to contain approximately the same percentage of ash as the apple. Most of this difference is undoubtedly due to contamination of the juice, for the silica contents of the samples of ash were determined, and it was found that the percentage of silica in the juice was about 15 times as great as in the whole apple. Thus the juice must have been contaminated by small chips of silica from the rough surface of the porcelain press, which was an old one. The figures for silica-free ash show such differences (within the limits of probable error) between the ash of the whole apple and the juice as might be expected if some solution of cell wall mineral matter had taken place, and it will be shown that there is much reason to believe that this has occurred. According to Loew [1899] the magnesia of plants is chiefly present in the cell wall in close association with lecithin, and he considers that magnesium is related to the assimilation of phosphates for the formation of lecithin and nucleoproteins. It is probable that calcium also may be present in the cell wall, most probably as a compound with pectin or pectic acid. When the juice is pressed out it passes through the cell walls, and it is obvious that some proportion of the mineral substances present there may be washed out, particularly when the juice is acid. If all the 98 g. of juice present in 100 g. of apple could be obtained by pressing, it should be expected that the total ash content of the juice and the whole apple would be the same, but only about 70 % of juice is obtained when killed apple tissue is pressed in the ordinary way; about 140 g. of fresh material are therefore required to give 100 g. of juice and if this juice contains material dissolved from the cell walls it may be derived from those of that weight of fresh apple. This would cause the ash content of the juice to be slightly higher than that of the whole apple.

It is interesting to see whether the figures for the individual constituents accord with this theory. It was not expected that any of the potash would be derived from the cell wall, and the figures in Table VII support this, for the differences between the percentages of potash in the juice and the whole apple are very small, and are within the limits of error. It thus seems likely that all, or nearly all, the potash of the apple is present in the cell sap. In this connection it may be mentioned that one analysis of the alcohol-insoluble residue

of the apple was made, and the residue from 100 g. of apple contained 0.0011 g. of potash; thus it is possible that a very small percentage of the potash, about 1 %, is present in the cell wall. This percentage may be even less, for, since potassium sulphate is insoluble in alcohol, some potassium sulphate originally in the sap may have been held in the residue.

It is difficult to discover whether any cell wall phosphorus could be dissolved by the juice, since the phosphorus in the form of lecithin would not be expressed by the juice, and this large difference between the amounts of phosphorus in the juice and the whole apple would mask any small difference that might occur owing to the solution of a small percentage of phosphate that might be present in the cell wall. It is probable, however, that most of the free phosphates of the apple as well as any hexosephosphate that may occur are present as potassium salts in the cell sap, and that the cell wall contains little more than the phosphate which is combined as lecithin. This conclusion is supported by the figures of Table VII, which show that the difference between the amount of phosphorus in the juice and in the whole apple is from 10 to 20 %, for this percentage of phosphorus as lecithin was previously found [Brown, 1927] in a preliminary determination of phosphorus compounds in apples. In the Dabinett apples, however, the second running of juice contains less phosphate than the first; this suggests that the first running may have dissolved some phosphate from the cell wall, but this single result needs confirmation.

The figures for magnesia, however, afford much support to the theory of the solution of cell wall material by the juice, for the magnesia content of the juice is from 15 to 40 % higher than that of the whole apple. The maximum figure is that which would be obtained if none of the magnesia remained in the cell wall, for the weight found in 100 g. of juice corresponds with that from 140 g. of fresh material. The calcium was not estimated, but in the process of determining magnesia, calcium is first precipitated and it was noted that there was always a larger precipitate of calcium from the ash of the juice than from that of the whole apple. In some earlier work on Lane's Prince Albert apples from East Malling, given in Table II, it will be seen that a much larger percentage of lime was present in the juice than in the whole apple, which supports the evidence derived from the magnesia figures. It might be expected that the first runnings of juice would contain more mineral matter derived from the cell wall than the second running, and the figures of Table VII support this, although the differences are too small to be entirely conclusive.

It is interesting to observe the changes which took place during the 6 weeks' storage. The differences obtained in the samples stored for 4 and 6 weeks respectively must be almost entirely due to sampling, for the changes during a fortnight would hardly be detectable, and it is obvious that the small increase of acidity during the last two weeks of storage is due to individual variation. The mean of the two analyses after 4 and 6 weeks should therefore give an accurate measure of the state of the apple after 5 weeks' storage and

it is interesting to note that, although the properties of the juice have altered, the percentage of mineral constituents in the fresh apple remains unchanged, showing that the sampling error has been reduced by using half-apples. The changes in the composition of the juice are considerable, acidity and specific gravity have fallen and p_H has increased correspondingly; these are normal effects of storage. On the other hand, it is to be noted that the percentages of phosphate and magnesia in the juice are less, and this agrees well with what has already been deduced as to the solution of the mineral constituents of the cell wall by juices containing acid.

SUMMARY.

1. A method of calculating the value of "equivalent potash" content of apple juice from titration and p_H values is suggested.

2. Measurements of titration, p_H and "equivalent potash" are given for samples of Lane's Prince Albert and Cox's Orange Pippin picked at the normal season and also earlier and later. Similar measurements on samples from a single picking of other kinds of apple are also given.

3. A comparison has been made between the mineral content of apples and that of their expressed juice on two varieties of apples.

The same percentage of potash was found in the expressed juice as in the whole apple, indicating that most of the potash of the apple is present in the cell sap. Larger percentages of magnesia and lime were found in the juice than in the whole apple, from which it is concluded that the juice has dissolved lime and magnesia from the cell wall. There is some evidence that this solution of cell wall material by the juice varies with the acidity of the juice.

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