settle. The other specimens retained their shape. Furthermore these *Eucommia* specimens left no flakes of undissolved material like the Ceylon specimens. It is possible that these flakes consist of foreign matter, since the specimens of crude rubber used are no doubt less free from foreign material than the extracted sample from *Eucommia.*

It is evident that the literature dealing with the solubility of caoutchouc, especially the solubility in ether, is confusing. It is further evident that investigators of the elastic constituent of *Eucommia ulmoides* have not determined clearly the exact nature of that substance. Its solubility in ether is clearly established in the foregoing experiments, and yet Weiss considered the substance to be caoutchouc, and not viscin, because it only swelled up and did not dissolve in ether. Again, two genuine samples of crude rubber were found to be soluble in ether, not as rapidly as carbon disulfide perhaps, but still distinctly soluble.

It is possible that all the confusion is due to variation in the specimens used by the various investigators. Crude rubber, for instance, comes from several different sources, and the coagulation of the latex in the preparation of such rubber is not always accomplished in the same way. As regards the material from *Eucommia* it must be remembered that the foregoing notes deal entirely with the elastic constituent from the bark. The leaves, twigs and smaller branches may contain a principle of somewhat different chemical properties. In complex substances like caoutchouc, age, for instance, probably has an important modifying influence. If it were possible to secure material from all parts of the individual tree, and study the elastic principle with reference to its solubility in organic solvents, the results might go far towards removing the confusion which at present exists.

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY OF THE UNIVERSITY OF NEBRASKA EXPERIMENT STATION.]

ON THE DUCLAUX METHOD FOR THE ESTIMATION OF THE VOLATILE FATTY ACIDS.

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The Duclaux[1] method for the determination of the volatile fatty acids is based upon the fact that each of the acids of the series $C_nH_{2n+1}COOH$ has a constant rate of vaporization when distilled under given conditions. For example, if a definite volume of a dilute solution of any one of the lower fatty acids is subjected to distillation and the distillate collected in 10 cc. fractions, the amount of acid in any one fraction will bear a definite relation both to the total amount of acid in the original

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[1] Duclaux, *Ann. chim. phys.*, 2, 289 (1874); *Ann. inst. Pasteur*, 9, 265 (1895).

solution and to the amount of acid remaining in the flask at the time of collecting that fraction. Duclaux showed that each of the first five acids of the series $C_nH_{2n+1}COOH$ has its own definite rate of distillation when distilled under these conditions. He determined a set of constants for each acid by distilling 110 cc. of an approximately 2% solution of each acid, collecting the distillate in 10 cc. fractions and titrating each fraction. The set of constants then represents the percentage of acid in each fraction in terms of the acidity of the total distillate. Duclaux furthermore showed that these constants could be made use of in determining the amounts of the various acids in unknown mixtures of two or more of them. He *assumes* in this, that each acid in a mixture behaves as if it were alone and follows its own law of distillation. Duclaux made use of his method in determining the acids in wine.

Other investigators have made use of Duclaux's method in quantitatively estimating the acids formed in various fermentations such as those taking place in silage and in the ripening of cheese. Dox and Neidig,[1] and Hart and Williman[2] used the method in investigations of the acids formed in corn silage, while Jensen[3] and Suzuki, Hastings and Hart[4] have used it in determining the volatile acids produced in cheese. Richmond[5] has made a study of the method as applied to formic acid and acetic acids alone and in mixtures.

The above-named authors have by the use of this method, estimated the quantities of as many as four acids in a single mixture.

In the course of an investigation at the Nebraska Station of the acids formed during the fermentation of various silage mixtures, it became evident that the Duclaux method for the determination of the volatile fatty acids is not reliable and that the facts do not warrant its acceptance. From a critical study of the method we have come to the following conclusions:

1. Small variations in the experimental results, such as are within the limits of error of the method, may cause such wide variations in the calculated results as to make them of no quantitative value.

2. When more than two acids are present in an unknown mixture, as might be expected, several calculations may be made to fit the analytical results; that is, the results of a Duclaux series on an unknown mixture may be calculated in terms of one, two, three or more acids and furthermore in the case of three or more acids more than one com-

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[1] Dox and Neidig, *Iowa Exp. Sta. Res. Bull.*, 7 (1912).

[2] Hart and Williman, THIS JOURNAL, **34**, 1619 (1912).

[3] Jensen, *Landw. Jahr. Schweiz*, **18**, 319 (1904).

[4] Suzuki, Hastings and Hart, *J. Biol. Chem.*, **7**, 431 (1909).

[5] Richmond, *Analyst*, **33**, 305 (1907).

bination of the same acids may be calculated to correspond with the Duclaux series as determined.

3. Finally, the Duclaux method erroneously assumes that each acid will follow its own law of distillation even when present in a mixture. This tacitly assumes that the vapor pressure of one substance is not affected by the presence of other substances.

Experimental Part.

Our attention was first directed toward the redetermination of Duclaux's constants, for the first four fatty acids, under the following conditions: In each case the purest obtainable acids[1] were employed. In each case 75 cc. of 0.1 *N* acid was made up to a volume of 110 cc. and distilled from a 200 cc. side tube distilling flask. Care was taken to keep the stillhead heated to a constant temperature to prevent condensation in the upper part of the flask. The temperature was so regulated that 100 cc. distilled in approximately 45 minutes. The distillate was collected in 10 cc. fractions and titrated with 0.1 *N* $Ba(OH)_2$ using phenolphthalein as indicator. Under these conditions the following results were obtained:

Table I.—Formic Acid.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
1.......	3.17	3.40	3.59	3.87	4.16	4.66	5.16	6.06	7.23	9.70
2.......	3.08	3.40	3.55	3.93	4.11	4.59	4.95	6.31	7.07	9.67
3.......	3.00	3.31	3.52	3.84	4.08	4.59	5.07	5.98	7.11	9.75
4.......	2.95	3.31	3.49	3.88	4.15	4.55	5.08	5.95	7.10	9.64
A......	3.05	3.35	3.53	3.88	4.12	4.60	5.06	6.07	7.13	9.69
B......	3.05	6.40	9.93	13.81	17.93	22.53	27.59	33.66	40.79	50.48
C......	6.04	12.66	19.66	27.35	35.51	44.65	54.65	66.67	80.80	100
F......	4.04	8.53	13.24	18.41	23.90	30.04	36.78	44.88	54.38	67.30

Four distillations were made and Series 1, 2, 3 and 4 represent the titration figures, in terms of 0.1 *N* alkali, of the successive 10 cc. fractions. Series A represents the average of Series 1, 2, 3, 4, and B the sum of these figures for a given amount of distillate. Series C represents the figures of Series B in terms of the percentages of the total amount of acid distilling in 100 cc. This series is the one usually referred to as the Duclaux constants. In Series F are given the results of Series B calculated in terms of the percentages of the total amount of acid in the original mixture.

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[1] Samples of the formic and propionic acids used were boiled with excess of barium carbonate till neutralized, the resulting solution filtered and evaporated to dryness. Analyses of the salts thus obtained gave the following results:

0.7301 g. $Ba(CHO_2)_2$ gave 0.7479 g. $BaSO_4$
0.5564 g. $Ba(C_3H_5O_2)_2$ gave 0.4560 g. $BaSO_4$

	Theory.	Found.
$Ba(CHO_2)_2$	60.41% Ba	60.28% Ba
$Ba(C_3H_5O_2)_2$	48.47% Ba	48.23% Ba

Unfortunately the sample of acetic acid used in the experiment, was not saved. There is no reason to suppose that it was less pure than the formic and propionic.

In the following tables are given the results for acetic and propionic acids, obtained in exactly the same manner as those for formic acid above:

TABLE II.—ACETIC ACID.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
1.......	4.75	5.07	5.13	5.35	5.51	6.02	6.21	6.76	7.35	8.77
2.......	4.69	4.98	5.14	5.38	5.51	5.91	6.24	6.75	7.32	8.68
3.......	4.82	4.91	5.12	5.33	5.53	5.90	6.30	6.73	7.29	8.71
4.......	4.75	4.99	5.13	5.35	5.51	5.88	6.14	6.75	7.37	8.78
5.......	4.73	4.97	5.09	5.40	5.58	5.98	6.24	6.80	7.41	8.70
A......	4.75	4.98	5.12	5.36	5.53	5.94	6.22	6.76	7.35	8.73
B......	4.75	9.73	14.85	20.21	25.74	31.68	37.90	44.66	52.01	60.74
C......	7.82	16.01	24.44	33.27	42.37	52.14	62.39	73.52	85.79	100
F......	6.33	12.97	19.80	26.94	34.32	42.20	50.53	59.54	69.34	80.98

TABLE III.—PROPIONIC ACID.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
1......	8.44	8.21	7.87	7.76	7.32	7.22	6.73	6.44	5.84	5.33
2......	8.50	8.11	7.90	7.73	7.37	7.20	6.70	6.41	5.82	5.37
3......	8.41	8.07	7.85	7.78	7.32	7.18	6.68	6.44	5.79	5.38
4......	8.47	8.19	7.85	7.73	7.40	7.13	6.74	6.38	5.81	5.40
A.....	8.45	8.14	7.87	7.76	7.35	7.18	6.71	6.42	5.81	5.37
B.....	8.45	16.59	24.46	32.22	39.57	46.75	53.46	59.88	65.69	71.06
C.....	11.89	23.34	34.42	45.34	55.68	65.79	75.26	84.25	92.44	100
F.....	11.26	22.12	32.61	42.96	52.76	62.33	71.28	79.84	87.58	94.74

TABLE IV.—DUCLAUX CONSTANTS.

	Acid.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
Formic....	D. 1874	5.5	11.9	18.5	25.7	34.0	43.1	53.1	65.2	79.8	100
	D. 1895	5.9	12.2	19.0	26.4	34.4	43.2	52.8	64.6	79.6	100
	S. 1915	6.04	12.66	19.66	27.35	35.51	44.65	54.65	66.67	80.8	100
	P. 1916	5.04	11.00	17.58	24.78	32.91	41.69	51.92	64.19	79.07	100
Acetic....	D. 1874	7.5	15.4	23.0	32.0	41.2	50.8	61.0	72.5	84.9	100
	D. 1895	7.4	15.2	23.4	32.0	40.9	50.5	60.6	71.9	84.4	100
	S. 1915	7.82	16.0	24.4	33.2	42.4	52.1	62.4	73.5	85.8	100
	P. 1916	7.30	15.17	23.35	32.0	41.07	50.72	61.02	72.26	84.96	100
Propionic..	D. 1874	11.3	22.8	34.0	44.6	55.3	65.4	74.7	84.0	92.5	100
	D. 1895	12.1	24.0	35.3	46.2	56.8	66.7	76.2	83.0	93.0	100
	S. 1915	11.9	23.3	34.4	45.3	55.7	65.8	75.3	84.3	92.4	100
	P. 1916	12.16	23.75	34.85	45.66	56.14	66.12	75.65	84.63	92.68	100

Duclaux determined two sets of constants for the first five acids, one in 1874[1] and one in 1895.[2] These are given in Table IV (D. 1874 and D. 1895) together with two sets of constants determined by us (S. 1915 and P. 1916). Duclaux's two sets of constants agree more closely in the case of acetic and formic acids than with the others. A detailed

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[1] Duclaux, *Ann. chim. phys.*, **2**, 289 (1874).

[2] Duclaux, *Ann. inst. Pasteur*, **9**, 269 (1895).

inspection of the table brings out the fact that there is no close agreement among the different sets of constants. We believe these discrepancies arise from unavoidable experimental variations during the process of distillation. Other investigators have attributed them to such factors as condensation in the stillhead, unequal rate of heating, etc. These were eliminated as far as possible by surrounding the flask with an asbestos jacket to prevent condensation and carrying out the distillations at a uniform rate. We believe these variations are within the limits of error of the method. We arrived at these conclusions from an experimental study of known mixtures of different acids.

Expt. 1.—The first distillation was carried out according to the method of Duclaux on a mixture of 20 cc. of 0.1 *N* formic acid, 50 cc. of 0.1 *N* acetic acid and 40 cc. of distilled water, as shown on page 733, with the results as shown in Table V.

TABLE V.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
A......	4.07	4.24	4.40	4.67	4.96	5.19	5.63	6.12	6.94	8.42
B......	4.07	8.31	12.71	17.38	22.34	27.53	33.16	39.28	46.22	54.64
C......	7.44	15.22	23.26	31.81	40.88	50.39	60.69	71.89	84.59	100
D......	7.31	15.06	23.07	31.57	40.41	50.00	60.18	71.56	84.36	100
E......	6.98	14.35	22.14	30.40	39.04	48.41	58.37	69.82	83.02	..
F......	7.4	15.2	23.4	32.0	40.9	50.5	60.6	71.9	84.4	100

A gives the average titration figures of three distillations for each successive 10 cc. portion, B the sum of the successive titration figures, and C the same expressed in percentages of the sum of the titration figures for the ten fractions. D gives the series as calculated from the amounts of acid in the original mixture using our constants. The method of calculation as used by Duclaux and other investigators is a method of cut and try. It consists in selecting at random such proportions of acids as will when calculated in terms of the constants for the single acids give a series closely approximating the experimentally determined series. The unknown mixture is then considered to be made up of the acids in the proportions used in the calculation. The details of the calculation are as follows: Treating this case as an unknown, suppose that we have hit by chance upon the proportions of the two acids which were actually taken, namely $^2/_7$ and $^5/_7$. From our Duclaux constants (S. 1915) for formic acid we find that 6.04% distils in the first 10 cc. In the experiment since formic acid makes up $^2/_7$ of the mixture, $^2/_7 \times 6.04\%$ or 1.72% is the amount of formic acid in the mixture which comes over in the first 10 cc. In a similar manner $^5/_7 \times 7.82\%$ (our Duclaux constant for acetic acid) or 5.59% represents the amount of acetic acid distilling in the first 10 cc., $1.72\% + 5.59\% = 7.31\%$ which agrees fairly well with the experimental figure. The figures for the other fractions of Series D are obtained in the same way. Series E is calculated from the same proportion of formic and acetic acids, namely $^2/_7$ and $^5/_7$, but using Duclaux's own constants (D. 1895). An inspection of Table V shows that there is some variation between the calculated and the experimentally determined series.

If this method for determining the proportions of acids in unknown mixtures is reliable it ought to be possible to set up pairs of equations for any fraction, the solving of which will give us the proportions of acids in the mixture. If x represents the fractional part of formic acid and y the fractional part of acetic acid in the mixture, then $x + y = 1$.

Experimentally we find that 7.44% of the amount of acid distilling in the 10 fractions comes over in the first 10 cc. The constants for the first fraction for formic and acetic acids are, respectively, 6.04% and 7.82%. The equation will be $6.04\,x + 7.82\,y = 7.44$. Solving the pair of equations $x + y = 1$.

$$\begin{aligned} 6.04\,x + 7.82\,y &= 7.44 \\ x &= 21.35\% \\ y &= 78.65\%. \end{aligned}$$

Solving the equation of the third fraction, namely $19.66\,x + 24.44\,y = 23.26$ together with the equation $x + y = 1$, $x = 24.69$, $y = 75.31$. For the fifth fraction $35.51\,x + 42.37\,y = 40.41$.

$$\text{Solving } x = 28.57\% \qquad y = 71.43\%.$$

These equations lead to results varying between 21.3% and 28.6% of formic acid and 71.4 and 78.6% of acetic, whereas the original mixture contained 28.6% formic acid and 71.4% acetic. There is thus a maximum variation of about 7% between the theoretical and observed results. However, if we solve the simultaneous equations for any two fractions the variations are very much wider. For example, the equations for the first and third fractions give 1% formic and 99.0% acetic while the equations for the third and fifth fractions give 9.0% formic and 91.0% acetic.

Series E, Table V, is calculated for the mixture $^2/_7$ formic and $^5/_7$ acetic, using Duclaux's constants (D. 1895). This series shows a wide variation from the experimental Series C. In Series F are given Duclaux's constants for acetic acid. These agree so closely with the experimental Series C that had we been treating this mixture as an unknown, we would have concluded that it consisted of acetic acid only.

Expt. 2.—In Table VI are given the results of a distillation of a mixture 15 cc. 0.1 N propionic acid, 60 cc. 0.1 N acetic acid and 35 cc. distilled water.

TABLE VI.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
A......	5.25	5.45	5.58	5.76	5.87	6.10	8.32	6.73	7.25	8.38
B......	5.25	10.70	16.28	22.04	27.91	34.01	40.33	47.06	54.31	62.69
C......	8.38	17.07	25.9	35.16	44.52	54.21	64.33	75.06	86.64	100
D......	8.27	16.89	25.65	34.73	44.09	53.79	63.95	74.73	86.51	100
E......	8.44	16.96	25.78	34.82	44.08	53.74	63.72	74.12	86.12	100

Series A, B and C have here the same significance as in Table V. Series D is calculated from the proportions of acids in the original mixture, using our second set of constants (P. 1916). Series E again is calculated from Duclaux's constants (D. 1895). The two calculated series are in closer agreement with each other and with the experimental series than was the case with the formic, acetic mixture.

Calculated by means of simultaneous equations the per cent. of acetic varies from 21.8% to 23.3% and the propionic from 78.2% to 76.7%, whereas the mixture contained 20% and 80%.

There is a greater difference between the constants for acetic and propionic acids than between those for formic and acetic. This explains why there is better agreement between the calculated and observed results in the case of the former mixture than the latter.

Expt. 3.—A mixture of 50 cc. 0.1 *N* propionic, 20 cc. 0.1 *N* acetic 5 cc. 0.1 *N* formic acid and 35 cc. distilled water was subjected to the Duclaux distillation. The results are shown in Table VII.

TABLE VII.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
A.....	7.14	6.91	6.85	6.73	6.65	6.52	6.48	6.46	6.46	6.63
B.....	7.14	14.05	20.90	27.63	34.28	40.80	47.28	53.74	60.20	66.85
C.....	10.69	21.02	31.27	41.34	51.29	61.05	70.73	80.42	90.07	100
E.....	10.37	20.60	30.62	40.63	50.58	60.38	70.16	79.97	89.72	100
F......	10.44	20.86	31.04	41.09	51.06	60.82	70.48	78.82	89.82	100

Again A, B and C have the same significance as in Table V and E is the series calculated from the proportions of the three acids in the original mixture using our constants (P. 1916). Series F is calculated from the proportions of the three acids using Duclaux's constants (D. 1895). It agrees more closely with the experimental Series C than does our Series E. The calculated and observed series agree quite well. If the results are calculated by means of three simultaneous equations they show no sort of agreement with the original mixture. Basing the equations on the first and second fractions, the results give formic acid 73.1%, acetic 77.7% and propionic 104.6%; the third and fourth fractions give formic 18.4%, acetic 3.5%, propionic 78.1%; the second and fifth fractions give formic 6.6%, acetic 22.0% and propionic 71.4%, while the sixth and seventh fractions give formic 18.8%, acetic 1.5% and propionic 78.7%. The original mixture contained formic 6.66%, acetic 26.66% and propionic 66.66%.

Suppose the above mixture had been an unknown. A mathematical calculation as above gives no results which are capable of interpretation. By the method of cut and try several series agreeing more closely with the experimental one may be calculated using entirely different proportions of acids. Some results of such calculations are given in Table VIII.

TABLE VIII.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
C.....	10.69	21.02	31.27	41.34	51.29	61.05	70.73	80.42	90.07	..
E.....	10.37	20.60	30.62	40.63	50.58	60.38	70.16	79.97	89.72	..
F......	10.44	20.86	31.04	41.09	51.06	60.82	70.48	78.82	89.82	..
M.....	10.50	21.05	31.31	41.47	51.49	61.28	71.10	79.32	90.12	..
N.....	10.74	21.20	31.39	41.48	51.49	61.23	70.90	80.54	89.95	..

Series C is the experimental series of Table VII. Series E is calculated from the original mixture which consisted of formic 6.66%, acetic 26.66% and propionic 66.66% using our constants, and Series F is calculated from the same using Duclaux's constants (D. 1895). Series M is calculated by the cut and try method from *66.66% propionic* and *33.33% acetic, leaving out the formic entirely,* while Series N is calculated from *20% formic and 80% propionic, leaving out the acetic acid.*

Expt. 4.—A mixture containing 26.66% formic, 66.66% acetic and 6.66% propionic acids was subjected to distillation with the results as shown in Table IX.

TABLE IX.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
A......	4.64	4.74	4.94	5.07	5.36	5.66	6.00	6.46	7.36	8.85
B......	4.64	9.38	14.32	19.39	24.76	30.42	36.42	42.88	50.24	59.09
C......	7.85	15.87	24.23	32.79	41.90	51.48	61.63	72.56	85.02	100
D......	7.61	15.60	23.82	32.49	41.41	51.06	61.18	72.40	84.99	100
E......	7.70	15.77	24.11	32.82	41.82	51.53	61.67	72.80	85.28	100

In Table IX Series A gives the average figures for four distillations. A and B have the same significance as in the preceding ones and C is again the experimental series representing the percentage of acid distilling at any one point, in terms of the total acid distilling in the ten fractions. Series D is again calculated from the proportions of the three acids in the original mixture using our constants (S. 1915). In Series E is given a set of results as calculated by the method of cut and try, assuming that the mixture contained *15.38% formic instead of 26.66%, 80.8% acetic instead of 66.66% and 3.82% propionic instead of 6.66%.*

Expt. 5.—A mixture containing 15 cc. of 0.1 *N* formic, 50 cc. 0.1 *N* acetic, 5 cc. 0.1 *N* propionic, 5 cc. 0.1 *N* butyric acid and 35 cc. distilled water was subjected to the Duclaux distillation. The total acidity of this mixture is, therefore, made up of 20% formic, 66.66% acetic, 6.66% propionic and 6.66% butyric. The data is given in Table X.

TABLE X.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
A......	5.15	5.24	5.29	5.43	5.55	5.78	6.02	6.43	7.08	8.34
B......	5.15	10.39	15.68	21.11	26.66	32.44	38.46	44.89	51.97	60.31
C......	8.54	17.23	25.99	35.00	44.20	53.78	63.77	74.43	86.23	100
D......	8.43	16.99	26.32	34.68	43.76	53.46	63.15	74.00	86.05	..
E......	8.57	17.33	26.18	35.24	44.12	53.90	63.75	74.06	85.92	..
F......	8.39	17.04	25.88	35.08	44.23	53.80	63.82	74.22	86.16	..

Again A, B and C have the same significance as in preceding tables. D is calculated from the original mixture using our constants (S. 1915) except in the case of butyric acid where Duclaux's constant was used. Series E is calculated by the cut and try method using Duclaux's constants from a mixture of formic 10%, acetic 70%, propionic 13% and butyric 7%. Series F is calculated from a mixture of acetic 79% and propionic 21%, assuming that formic and butyric acids are absent. It agrees more closely with the experimental series than either of the other two.

Our results show conclusively that, whereas the Duclaux method will give approximate results on mixtures containing two known acids, it is of no value for examining mixtures in which both the number of acids present and their relative proportions are unknown. This is based not only on our own experimental work but holds true in the case of the results of other investigators. Table XI is a repetition of Dox and Neidig's Table I[1] giving the results obtained in the distillation of a sample of acids obtained from corn silage.

TABLE XI.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
A......	4.6	4.75	4.95	5.2	5.45	5.65	6.05	6.6	7.4	8.85
B......	4.6	9.35	14.3	19.5	24.95	30.6	36.65	43.25	50.65	59.5
C......	7.73	15.7	24.03	32.77	41.93	51.43	61.59	72.69	85.1	100
D......	7.78	15.86	24.26	32.98	41.9	51.5	61.50	72.63	84.9	100
	130 A,	4 P,	1 B,	1 V						
E......	7.77	15.88	24.30	33.05	42.06	51.61	61.64	72.64	84.92	100
	2 F,	87 A,	4 P,	2 B						

Series C is their experimentally determined series and D is calculated by the cut and try method and corresponds to a mixture of 130 parts acetic acid, 4 of propionic, 1 of butyric and 1 valeric. Series E was calculated by us and corresponds to an entirely different mixture, namely, 2 parts formic, 87 acetic, 4 propionic and 2 butyric and agrees as closely

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[1] Dox and Neidig, *Iowa Exp. Sta. Res. Bull.*, **7**, 14 (1912).

with Dox and Neidig's experimental series as does their own calculated series.

Table XII is reconstructed from Dox and Neidig's Table II, which was calculated from the results obtained in the distillation of another acid mixture obtained from corn silage.

TABLE XII.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.	80 cc.	90 cc.	100 cc.
A......	2.65	2.8	2.9	3.05	3.15	3.35	3.6	3.95	4.5	5.55
B......	2.65	5.45	8.35	11.4	14.55	17.9	21.5	25.45	29.95	35.5
C......	7.46	15.35	23.51	32.1	40.98	50.42	60.56	71.7	84.36	100
D......	7.4	15.2	23.4	32.0	40.9	50.5	60.6	71.9	84.4	100
		acetic acid only								
E......	7.45	15.32	23.52	32.11	41.01	50.55	60.6	71.76	84.35	100
	8 formic, 83 acetic, 4 propionic									

Their experimental Series C agrees so closely with the Duclaux series for acetic acid, which is given in D, that they concluded the mixture contained acetic acid only. Series E as calculated by us is practically identical with their experimental Series C and is based on 8 parts formic acid, 83 acetic and 4 propionic. Thus not only may the results be calculated in terms of different acids but results which seem to indicate the presence of one acid may be calculated just as well in terms of three acids.

Duclaux as well as the other investigators who have used this method make it a means for determining not only the *quantities* of acids, but also the *kinds* of acids, present in an unknown mixture. Calculations are carried out by the cut and try method, using different acids in varying proportions until a series of figures agreeing closely with the experimental series is obtained. The solution under examination is then considered to be made up of the particular acids in the proportions which give the desired series of figures. Obviously such a method is of no value, either quantitatively or qualitatively when not only *different proportions* of the same acids but *different acids* may give identical series of figures. For example, Suzuki, Hastings and Hart[1] from a Duclaux distillation on a mixture of acids obtained from cheese concluded that the mixture was made up of acetic 96.0%, propionic 2.5% and butyric 1.5%. Their figures are given in Table XIII.

In the first column is given their experimental series and in the second is given their calculated series which is based on 96% acetic, 2.5% propionic acid and 1.5% butyric. In the third column is given a series which we have calculated from 97.25% acetic and 2.75% butyric. We are just as much justified in concluding that the mixture contains acetic

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[1] Suzuki, Hastings and Hart, *Wis. Exp. Sta. Res. Bull.*, 11, 135 (1910); *J. Biol. Chem.*, 7, 437 (1909).

and butyric acids only as are Suzuki, Hastings and Hart in concluding that it is made up of acetic, propionic and butyric.

TABLE XIII.

Found.	Calculated for: A, 96%; P, 2.5%: B, 1.5%.	Calculated for: A, 97.25; B, 2.75.
7.70	7.67	7.68
15.65	15.69	15.70
23.84	24.00	24.00
32.40	32.77	32.76
41.56	41.74	41.71
51.10	51.33	51.29
61.49	61.37	61.30
72.86	72.53	72.51
85.20	84.80	84.74
100.00	100.00	100.00

Any series which is calculated, assuming that propionic acid makes up 10% or less of the mixture, may just as well be calculated with the propionic acid equally distributed between the acetic and butyric acids. In no case would this bring about a variation greater than 0.27 in the calculated figures. Investigators have often passed results in which the variations between the experimental and the calculated series are considerably greater than this. This situation arises from the fact that the Duclaux constants of acetic acid are approximately as much lower than those for propionic as the butyric constants are higher than those for propionic. These differences are given in Table XIV.

TABLE XIV.

1. Diff. A. & P.	2. Diff. P. & B.	3. Diff. 1 and 2.	4.
4.7	5.5	+0.8	+0.04
8.8	9.6	+0.8	+0.04
11.9	12.2	+0.3	+0.01
14.2	13.8	—0.4	—0.02
15.9	13.8	—2.1	—0.10
16.2	12.8	—3.4	—0.17
15.6	10.3	—5.3	—0.27
11.1	9.5	—1.6	—0.08
8.6	4.4	—4.6	—0.23

Suppose a series of figures had been calculated from a mixture assumed to contain as much as 10% of propionic, and suppose that this amount of propionic acid had then been calculated equally in terms of acetic and butyric acids. The differences which this would make in the series is shown in Col. 4.

A set of figures calculated, assuming the presence of any acid in small amounts, 5% or less, can just as well be calculated without that acid. Consequently results which assume the presence of any of the acids in small amounts have no meaning. Dox and Neidig give results which assume the presence of valeric and butyric acids in amounts as low as 0.74% and Suzuki, Hastings and Hart have drawn conclusions based on results which indicate the presence of several acids in proportions of less than 5%.

Finally, one of the sources of error in the method arises from the fact that substances do not behave the same when distilled from a mixture as when distilled from pure solutions. The method is therefore not based on sound theoretical principles.

Summary.

1. Our experiments show that unavoidable variations in experimental results, while small, may be sufficient to vitiate the results.
2. When more than two acids are present in a mixture practically identical series may be calculated from mixtures of different acids in varying proportions.
3. Results which indicate the presence of one acid may just as well be calculated in terms of three or more acids.
4. Small amounts of acids may be distributed just as well between the acids next higher and lower in the series.
5. The theory of the method is not sound.
6. The method, therefore, does not deserve either quantitatively or qualitatively for determining the composition of unknown mixtures of fatty acids.

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THE DETERMINATION OF GELATINIZATION TEMPERATURES OF STARCHES BY MEANS OF AN ELECTRICALLY HEATED CHAMBER ON THE MICROSCOPE STAGE.

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It has long been known that starches prepared from different species of plants show differences not only in microscopic appearance but also in gelatinization temperature. There is some evidence also that starches prepared from different varieties or strains of the same species vary in this respect. The differences in many cases are so slight, that in order to serve as a means of identification the determinations must be made by a method capable of giving duplicates that check within a fraction of a degree.

The method commonly used for determining the gelatinization temperature of starch consists in heating small samples with water in test tubes immersed in a water bath. The temperature is gradually raised and a sample removed for microscopic examination with every rise in