

THE ESTIMATION OF TANNIN IN CIDER.

By C. W. SPIERS, B.Sc.

(*Bio-Chemical Laboratory, Chemical Department, University of Bristol.*)

THE method at present in use for the estimation of tannin in apple juice and cider is that used by Lloyd¹. It is a modification according to Kraus² of Löwenthal's³ method, and consists in titrating the cider directly with potassium permanganate in presence of indigo as an indicator. The permanganate is standardized by means of "pure tannin," it being assumed that both this and the tannin present in the apple are digallic acid, and have the composition $C_{14}H_{10}O_9$. It is apparent that all the permanganate reducing substances present in the cider will be included in the tannin content; moreover the amount of indigo used is not sufficient to retard noticeably the oxidation of the non-tannins for which purpose it was originally used in Löwenthal's method. The results obtained by this method are therefore only comparative.

Gravimetric methods, such as the hide powder method and Wislicenus'⁴ "sprouted alumina" method, which depend on the difference in weight of the residue obtained on evaporation before and after detannizing with the above named detannizing agents, are in our case of little value, since it is practically impossible to obtain a residue of constant weight by evaporation of the cider.

The methods in use by botanists are also open to criticism, and thus not suitable for the investigation of tannic acid in cider. Sanio's⁵ method, which is based on the assumption that tannins are quantitatively precipitated by potassium dichromate, does not give trustworthy

¹ Lloyd, Report on researches on Cider making (Board of Agriculture), 1903.

² G. Kraus, *Grundlinien zu einer Physiologie des Gerbstoffs*, Leipzig, 1889.

³ Löwenthal, *Zeit. für Anal. Chem.* 1866, 5, 838; 1877, 16, 33; 1881, 20, 91.

⁴ Wislicenus, *Collegium*, 1905, 85; 1905, 213; 1905, 23; 1906, 316; 1907, 157.

⁵ Sanio, *Botanische Zeitung*, 1863, 17.

results as was shown by Drabble and Nierenstein¹, since gallic acid is also precipitated by potassium dichromate. The same objection applies to a method of Kraus² based on potassium dichromate precipitation. Fleck³ precipitates the tannin by means of neutral copper acetate, weighing the copper oxide resulting from the ignition of the precipitate. Sonnenschein⁴ uses Fehling's solution, also weighing the copper oxide. Both these workers deduced a relation between copper oxide and "pure tannin" in order to express their results. As will be shown later, such a relation cannot be used with any certainty. The colorimetric method of Jean⁵ depends upon a comparison of the tint produced by addition of ferric chloride to the solution to be examined with that produced by known concentrations of "pure tannin."

Such a comparison assumes that all plant tannins have the same constitution as gallotannic acid, which is obviously incorrect, since tannins can be divided into two groups giving green and blue colorations respectively with ferric chloride. Moreover it does not follow that solutions of different tannins which produce the same depth of coloration with ferric chloride are of the same concentration.

As was mentioned above, Kraus⁶ titrated solutions directly with potassium permanganate, including all reducing substances as tannin; the content of which was calculated by standardizing the permanganate with "pure tannin." It was assumed therefore that all tannin substances are "gallotannic acid." Neubauer⁷ also used potassium permanganate to estimate tannin in solutions of extracts, using, however, animal charcoal as a detannizing agent. The difference in the amount of permanganate used before and after detannizing gives a measure of the tannin present. For the standardization of the permanganate Neubauer used "pure tannin," and for convenience found a relation between "pure tannin" and oxalic acid with respect to their permanganate reducing value. The tannin content as found by this method would be somewhat too high; since other substances, notably gallic acid, are also absorbed by animal charcoal. Neubauer, however, realized

¹ Drabble and Nierenstein, *Biochem. Journ.* 1907, **2**, 96.

² Kraus, *Grundlinien zu einer Physiologie des Gerbstoffs*, Leipzig, 1889, p. 67.

³ Fleck, *Deutsche Gerbzeitung*, 1860, **3**, 14.

⁴ Sonnenschein, *Jahresbericht für Pharmazie*, 1868, p. 150; *Dingler's Polytech. Jl.* 1885, **256**, 555.

⁵ Jean, *Archiv der Pharmazie*, 1885, **214**, 992; *L'Union Pharmac*, 1880, **29**, 548.

⁶ Kraus, *loc. cit.*

⁷ Neubauer, *Zeit. für Anal. Chem.* 1871, **vol. 9**, 1.

that this method could only give the tannin content of the solutions analysed in terms of the tannin used to standardize the solution. In his own words: "Es entsteht daher die Frage: dürfen wir bei der Gerbstoffbestimmung der Eichenrinde das Tannin zum Vergleich zu Grunde legen? Strenge genommen allerdings nicht, wenn es allein darum ankäme den absoluten Gehalt der Rinden am Eichenrindengerbstoff zu bestimmen, denn es fehlt uns ja bei der volligen Unkenntnis des letzteren in absolutem reinem Zustande all' und jede Garantie das beide Gerbsauren gleichen Mengen von Chamaleon zur Oxydation bedürfen."

Of the precipitation methods involving the use of organic bases, that of Trotman and Hackford¹ was tried. This depends upon the precipitation of the tannin by strychnin in dilute alcoholic solution. Trotman and Hackford considered the compound which they prepared from "pure tannin" to have the composition $C_{14}H_{10}O_9 \cdot C_{21}H_{22}N_2O_3$. It will be shown that commercial "pure tannins" are mixtures which vary in composition and therefore do not give a strychnin compound of constant composition. Trotman and Hackford calculated their results on the basis of the above strychnin compound, *i.e.* they assumed tannic acid to be digallic acid. Nierenstein² has shown this to be incorrect, and considers tannic acid to be a complex molecule of digallic and leucodigallic acids. E. Fischer and Freudenberg³, on the other hand, have brought forward some evidence to show that tannic acid is penta digalloyl-glucose.

It has been found that Trotman and Hackford's method does not give consistent results with gallotannic acid owing to the solubility of the strychnin compound in the dilute alcohol used, and for this reason it is not suitable at present as a standard for the case of the tannic acid in cider. The method finally adopted was a modification of that of Körner and Nierenstein⁴ which consists in detannizing the solution to be analysed by means of casein, the total solids being estimated before and after detannizing. In this case, however, the tannin removed was determined by the difference in the permanganate titrations as in the method of Neubauer⁵ previously mentioned, *the casein removing nothing but the tannin from the cider or tannin solution analysed*, as shown by the work of Nierenstein⁶.

¹ Trotman and Hackford, *Jl. Soc. Chem. Ind.* 1905, 1097.

² Nierenstein, *Annalen*, 1912, 388, 223.

³ Fischer and Freudenberg, 1912, B. 45, 915; 1913, B. 46, 1116.

⁴ Körner and Nierenstein, *Chem. Zeit.* 1911, vol. 36, 31.

⁵ Neubauer, *loc. cit.*

⁶ Körner and Nierenstein, *loc. cit.*; Nierenstein, *Annalen*, *loc. cit.*

In the method here described a solution of potassium permanganate of about 5 grammes per litre concentration is made up and used as a stock solution. This is diluted to five times its volume as required, since such dilute solutions do not keep well. A solution of indigo carmine in dilute sulphuric acid, concentration about 5 grammes per litre, is made up: the titration is performed as in the method of Löwenthal¹. 20 c.c. of such a solution require approximately 10 c.c. of the diluted permanganate. The titration is carried out as in the method of Löwenthal as follows: 5 c.c. of the cider or tannin solution (0.3–0.5%) are added to 750 c.c. of water in a 1 litre shallow porcelain dish together with 20 c.c. of the indigo solution of which the permanganate titration is known.

The amount of permanganate required by the indigo compared with that used by the tannin is smaller in proportion than that suggested by some earlier workers, who used an amount of indigo which would require more permanganate than the tannin solution itself.

Neubauer observed on this point: "...bemerke ich nur dass die Indigo-lösung immer ein solche Konzentration haben muss dass 20 c.c. derselben allerwenigstens eine gleiche Menge Chamaleon wie das Tannin, oder sicherer noch die Hälfte mehr wie diese verlangen." It was found in the course of this work that quite reliable results were obtained if the fixed amount of 20 c.c. indigo solution were used. Larger amounts did not influence the volume of permanganate used by the tannin beyond the limits of experimental error. The end-point was, however, more difficult to determine with larger or smaller volumes of indigo solution.

The permanganate is run in slowly, at first about 1 c.c. at a time, with vigorous stirring for 5–10 seconds between each addition. When the blue colour of the indigo begins to disappear the permanganate is run in more and more slowly; and finally drop by drop until the liquid becomes a clear golden yellow with a tinge of pink round the edge of the dish. The appearance of this pink tinge is the most certain indication of a sharp end-point.

The titration cannot be carried out in artificial light and is best done in diffused daylight when the error is about 0.2 c.c. In actual practice at least three titrations should be made.

The detannizing of the solution is effected by shaking with casein, which to be efficient must be quite fat-free. For this work Kahlbaum's pure casein (nach Hammarsten) was used, previously extracted 36 hours

¹ Löwenthal, *loc. cit.*

with ether. It was found that for solutions of commercial "pure tannins" up to 0.5% concentration, detannizing was completely effected by shaking 50 c.c. with two quantities of one gramme of casein for 15 minutes. The liquid is filtered before the second addition of casein; and finally before titration through a barium sulphate filter, after which 5 c.c. are titrated as before.

The permanganate used was first standardized by means of Schering's tannin leviss puriss.

It was afterwards found that different samples of the same tannin gave somewhat different figures with the permanganate, since the commercial "pure tannins" are not homogeneous substances.

A number of pure tannins were therefore titrated and the average value taken.

Tannins purified by Nierenstein's and by Fischer's methods were included for comparison.

Table I contains the tannin value of the permanganate and the tannin content of the various samples used as found by detannizing the solutions with casein. The water present in the tannin was estimated in all cases by drying to constant weight at 40° C. in a vacuum desiccator containing calcium chloride. Duplicate determinations by this method usually differed by less than 0.05%. The dried samples were afterwards used for duplicate titrations with permanganate.

For convenience in standardizing the permanganate in the future the solution used was titrated against ammonium oxalate, which is easily obtained pure and is neither hygroscopic nor efflorescent.

From this it was found, using the average value of the tannins as in Table I, that:

1 gramme ammonium oxalate = 0.4648 gramme tannin.

Table II contains the results of analyses of various ciders; mostly of the bittersweet variety. The tannin content given by direct titration with permanganate is included for comparison. These, however, are probably somewhat lower than those found by Lloyd's method, since the small amount of indigo used by him would tend to make the tannin content appear larger by the inclusion of other oxidizable substances. The titration figures given here are those of the diluted permanganate. It should be noted that since the constitution of the cider-tannin is unknown, the amount of it present in the cider is expressed in terms of the tannin used to standardize the permanganate.

