The Determination of Tartaric Acid in Foodstuffs

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THE Agricultural Produce (Grading and Marking) (Cider) Regulations, 1930, introduced under the Agricultural Produce (Grading and Marking) Act, 1928, adopts certain standards for the two grades of cider, "Select" and "Select Cider, Champagne process." The "Select Champagne Process" grade may not contain any acid foreign to apples, and the "Select" grade may not contain more than 1 grm. per litre of tartaric or citric acids, other acids foreign to apple juice being prohibited. For the examination of ciders under these regulations, it became necessary to devise methods for the detection and estimation of these small quantities of acids in presence of malic acid. Of the methods for the determination and detection of tartaric acid which have been studied, the most promising appeared to be:

(*a*) The method having as its basis the comparative insolubility of potassium hydrogen tartrate in dilute acetic acid in presence of alcohol, or alcohol and ether.

(*b*) The method of Kling,[1] which depends on the insolubility of calcium racemate in dilute acetic and hydrochloric acids.

Method (*a*) forms the basis of the French official method adopted in the *Journal Officiel du* 22 *jan.*, 1907, an account of which (by Kling) appears in *Expertises Chimiques*, Vol. III, p. 185, and it also forms the basis of the American official method in *Methods of Analysis, A.O.A.C.*, 1925, p. 366, and in the 3rd edition, 1930, p. 141.

Method (*b*) is applicable only to *d*-tartaric acid. From an examination of commercial samples of tartaric acid derived from many sources I am able to state that all commercial tartaric acid on the market in this country appears to be exclusively the *d*-acid. Briefly, this method consists in adding to the solution containing *d*-tartaric acid, a solution of calcium acetate in dilute acetic acid, and a quantity of *l*-ammonium tartrate, which, while ensuring complete precipitation of the original *d*-acid as calcium racemate, will not cause precipitation of calcium *l*-tartrate. No precipitate, therefore, will be given in absence of *d*-tartaric acid, or acids whose calcium salts are insoluble in dilute acetic acid. Meso-tartaric acid is not precipitated as the calcium salt if the conditions given in the method are adhered to, but it has been found that in presence of *d*-tartaric acid, a lengthened period of standing with the reagents will result in the precipitation of some meso-tartrate. Racemic acid is, of course, precipitated as the racemate without addition of *l*-ammonium tartrate, and, if present, may be precipitated by adding the calcium reagent alone. It should be noted here, that the amount of *d*-tartaric acid originally present, and converted into racemate, will be one-half the amount found as calcium racemate. Oxalic acid, if present, will be precipitated by the calcium reagent, and should be eliminated before proceeding to the determination of *d*-tartaric acid.

The methods (*a*) and (*b*) referred to above have been studied by Hartmann and Hillig,[2] and with certain modifications have been adopted as tentative methods in America (*Methods of Analysis*, *A.O.A.C.*, 3rd edition, 1930, p. 271). Further study of the methods seemed necessary, owing to the proportionately large amount of malic acid present in cider, and also in view of the possibility of inactive malic acid appearing on the English market in the future.[3] This acid is obtained by passing benzene vapour mixed with oxygen over a heated vanadium catalyst, when maleic acid is produced. This gives malate on boiling with dilute alkali. Inactive malic acid is reported to be very pleasant to the palate, and may figure largely in beverages, provided it can compete commercially with the biochemical method of obtaining citric acid from cane sugar.[4]

The American official method for the determination of tartaric acid was found to give misleading results when a large proportion of malic acid was present. It should be borne in mind that the amount of malic acid present in cider is often five times the maximum amount of tartaric acid allowed by the regulations in the "Select" grade, and in one case a cider examined here was found to contain 7·5 grms. of malic acid per litre.

Hartmann and Hillig (*loc. cit.*) have considerably modified this official method, but their modification, also, is not satisfactory when a large excess of malic acid is present. These authors have estimated up to 0·15 grm. of tartaric acid in the presence of 0·1 grm. of malic acid and citric acid, and have found their method to give good results with this proportion of acids. It was found in experiments here that, if a reasonable titration for tartaric acid was desired, say, 5 ml. of $N/10$ alkali, representing 0·075 grm., the amount of malic acid which might be present in a portion of cider containing this amount of tartaric acid would sometimes be as much as 0·5 grm. A solution containing 0·1 grm. of tartaric acid, and 0·5 grm. of malic acid, when tested according to the method described (*loc. cit.*, p. 104), indicated the presence of 0·142 grm. of tartaric acid. High results were also given when inactive malic acid and tartaric acid were in the same solution; *e.g.* a solution containing 0·05 grm. of tartaric acid and 0·25 grm. of inactive malic acid indicated the presence of 0·074 grm. of tartaric acid. The investigation of this method was therefore abandoned.

The modification of Kling's method, referred to above, was then investigated, and was found to be satisfactory, even when the tartaric acid present was only one-seventh of the amount of a mixture of other hydroxy acids. At present, pure *l*-ammonium tartrate is somewhat expensive, and it is imperative to use the substance free from the *d*-salt. E. G. Kellett, of this department, has recently published[5] an improved technique for the preparation of the pure *l*-salt, which should greatly facilitate the cheapening of production, and so make more generally available the present modification of Kling's method, which has proved greatly superior to all other methods. For convenience, the technique which has been used in this laboratory for over a year, and has been found satisfactory, is given here.

PREPARATION OF SAMPLE.—In many cases the sample needs no special preparation, but in some preparations, such as jellies, jams, and other fruit products, sufficient sugar or colloidal material (such as gelatin or pectin) is present to make precipitation and filtration difficult. If a considerable quantity of alcohol is present, esters may have been formed, and saponification may be necessary before determining the acid. Solid matter, which could be oxidised by potassium permanganate, must be removed by preliminary filtration. Pectin, pectic acid and gelatin may be eliminated by adding excess of alcohol with a few drops of sulphuric acid, allowing the mixture to stand for half an hour, and filtering off an aliquot portion through a coarse fluted paper or a pad of cotton wool. In no case was sufficient pectin present in ciders examined here to require precipitation by alcohol, although in some instances filtration was slow. It is essential to obtain the free acids when pectin, etc., has to be eliminated, or when sufficient alcohol is present in the original sample to depress the solubility of calcium *l*-tartrate below its precipitating point under the conditions of the determination.

DETERMINATION.—Weigh or measure such a portion of the sample as will contain not more than 0·2 grm. of tartaric acid in the final aliquot portion, adjust to 35 ml. by dilution or concentration, add 3 ml. of *N* sulphuric acid, pour into a 250-ml. flask, rinse with 15 ml. of warm water and then with 96 per cent. alcohol, and make up to the mark with 96 per cent. alcohol. Industrial methylated spirits may be used in place of alcohol. Shake the mixture, and allow it to stand for half an hour, filtering if necessary. Transfer a convenient aliquot portion of the clear alcoholic solution so obtained into a centrifuge tube, and add a slight excess of neutral lead acetate solution. Shake vigorously for two minutes, and centrifuge for 15 minutes at about 1000 revs. per minute. Drain off the supernatant liquid thoroughly, and wash once with alcohol or methylated spirit, centrifuging and draining as before. Transfer the lead salts to a beaker with warm water, and pass in a rapid stream of hydrogen sulphide until the reaction is complete. Filter, wash thoroughly, boil the filtrate until it is free from hydrogen sulphide, and adjust the volume to 150 ml. Up to this point it has been assumed that the difficulties mentioned under "Preparation of sample" have been encountered; if they have not, take a portion of the sample, which will contain not more than 0·2 grm. tartaric acid, and dilute to 150 ml.

To the 150 ml. obtained by either process, add 15 ml. of reagent (*a*), 25 ml. of reagent (*b*) and 20 ml. of reagent (*c*), stir vigorously until calcium racemate begins to precipitate, and allow the mixture to stand overnight at room temperature. Filter by decantation on to a thin, lightly-tamped pad of asbestos, and transfer the precipitate to the crucible with a portion of the filtrate. Wash the contents of the crucible five times with water, filling the crucible about half full and sucking dry each time. Treat the precipitate and mat after removal from the Gooch crucible with 20 ml. of reagent (*d*), and wash the crucible thoroughly. Adjust the volume of solution to 150 ml. with water. Bring 50 ml. of reagent (*e*) to the boiling point and pour it through the Gooch crucible into the 150 ml. mentioned above, then bringing the temperature of the whole to 80° C. Cool, stir vigorously, and leave for at least 4 hours, stirring occasionally. Filter and wash as described in the first operation. Transfer the pad and precipitate to a beaker with 150 ml. of water, add 50 ml. of sulphuric acid, 10 per cent. by volume, and heat to 80° C. Immediately add standardised potassium permanganate solution until an excess is indicated. Again heat to 80° C., add an additional 5 ml. of the permanganate solution, and allow the beaker to stand for about 1 minute. After re-heating to 80° C., immediately add 10 ml. of the standard oxalic acid solution, and titrate back with the permanganate solution. One ml. of the permanganate solution equals 2·5 mgrms. of *d*-tartaric acid.

REAGENTS.—(*a*) Di-ammonium citrate. Dissolve 29 grms. of citric acid in about 200 ml. of water, carefully neutralise to methyl red with ammonia, add 14·5 grms. of citric acid, and make up to 1 litre with water. This solution contains 50 grms. per litre.

(*b*) Ammonium *l*-tartrate. Dissolve 3·2 grms. of the salt, entirely free from *d*-tartrate, in water, add 1 ml. of commercial formalin as a preservative, and dilute to 200 ml.

(*c*) Calcium acetate. Dissolve 16 grms. of calcium carbonate in 120 ml. of glacial acetic acid diluted with sufficient water, make up to 1 litre, and filter.

(*d*) Hydrochloric acid. Dilute 34 ml. of pure acid (conc.) to 1 litre.

(*e*) Calcium and sodium acetate. Dissolve 5 grms. of calcium carbonate in 20 grms. of acetic acid and sufficient water, add 100 grms. of sodium acetate, make up the solution to 1 litre and filter.

(*f*) Potassium permanganate. Prepare a solution in water containing 6·9745 grms. in a litre. Standardise this against pure tartaric acid, employing the complete precipitation process as for the sample taken. One ml. of the permanganate = nearly 2·5 mgrms. of the *d*-tartaric acid originally present, or nearly 5 mgrms. of racemic acid.

(*g*) Oxalic acid. Prepare a solution containing 13·8793 grms. per litre and titrate against the permanganate solution.

EXPERIMENTAL.—Active and inactive malic acids and citric acid (0·5-grm.-portions) were dissolved separately in 150-ml. portions of water, and the process described above was followed. In no instance was any precipitate observed on standing overnight in the first part of the process on adding reagents (*a*), (*b*) and (*c*). Minute crystals were observed if the mixtures were allowed to stand for 48 hours. These crystals were filtered off, and the second part of the process was continued. In no case was any precipitate given, showing that at the concentration used, the acids could not be mistaken for *d*-tartaric acid. In view of the insolubility of calcium racemate in the reagents employed, it was somewhat surprising to find that inactive calcium malate was sufficiently soluble to give no precipitate.

The effect of the presence of inactive malic acid on the determination of *d*-tartaric acid was studied by precipitating 0·15 grm. of tartaric acid alone, and a similar quantity of this acid mixed with 0·5 grm. of inactive malic acid under the given conditions. The volumes of standard permanganate solution used were almost identical in the two cases, showing that there was no appreciable precipitate due to inactive malic acid. Ordinary *l*-malic acid, and citric acid under similar conditions, did not interfere with the determination of tartaric acid.

The process was then repeated with the following mixture of acids:—*d*-tartaric acid, 0·15 grm.; *l*-malic acid, 0·50 grm.; inactive malic acid, 0·25 grm.; citric acid, 0·25 grm.

The permanganate titration was equivalent to 0·153 grm. of tartaric acid, showing that, even with this large excess of other acids, the method was reasonably accurate.

Genuine ciders, kindly supplied by the National Fruit and Cider Institute, Long Ashton, were examined, and it was found that these gave no measurable titration which would indicate the presence of tartaric acid by the method described. Weighed quantities of *d*-tartaric acid were added to these ciders, and determined. The amounts obtained by titration were almost exactly equal to the amounts of tartaric acid added in each case.

QUALITATIVE TEST OF CIDERS.—Over 300 ciders have been tested qualitatively for tartaric acid as follows: To 50 ml. of cider were added 5 ml. of reagent (*a*), 8 ml. of reagent (*b*), and 7 ml. of reagent (*c*), and the mixture was stirred vigorously at intervals during 4 hours. Ciders which contained no extraneous tartaric acid gave no precipitate in this time. Three mgrms. of tartaric acid could readily be detected by the formation of a distinct precipitate which was identified as calcium racemate, under the microscope, by the typical rosette-like form of the clusters of crystals.[6]

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