# THE DETERMINATION OF MALIC ACID IN FOOD PRODUCTS.

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"The difficulty in securing concordant results in the determination of malic acid in food products by the present methods made a careful investigation of these methods seem imperative. Accordingly, the following work was undertaken to find out, first, what conditions were essential to the complete recovery of pure malic acid from its solutions, and, second, how these conditions were adapted to the separation of malic acid from the other substances with which it occurs in food products.

A 1 per cent. solution of Schuchhardt's malic acid was made up, 10 cc. of which were found to contain 0.09997 gram of pure malic acid by titration against N/10 sodium hydroxide, using phenolphthalein as indicator. This acid gave no residue on ignition and was free from citric, tartaric, succinic, and mineral acids, as proved by the corresponding qualitative tests.

Ten cc. of this solution were treated by the method given in This Journal, **26**, 1536, with 1 cc. of 10 per cent. calcium chloride solution and a slight excess of ammonia, made up to a volume of 20 cc. with water, 60 cc. of 95 per cent. alcohol added, warmed and left over night, filtered, washed with 75 per cent. alcohol, ignited and titrated with *N*/10 hydrochloric acid. This gave 0.1909 gram malic acid or 190.9 per cent. of that taken. This must be due to the ammonia taking up carbon dioxide from the air and hence causing the precipitation of calcium carbonate. The reagents alone gave an abundant precipitate by this method. When the modified method recommended on p. 14, *Bull.* **105**, *Bureau of Chemistry*, omitting the ammonia, was used, no precipitate whatever was obtained. Hence any free malic acid, existing in food products, would not be precipitated owing to the fact that malic acid is not strong enough to separate the calcium from its combination with a mineral acid. Accordingly another precipitant was sought. Calcium acetate was substituted for the calcium chloride with the following results. Ten cc. of the solution of malic acid were taken, 2 cc. of 10 per cent. calcium acetate[1] solution, 8 cc. of water and 60 cc. of 95 per cent. alcohol were added, the solution was warmed and left over night, filtered, washed with 75 per cent. alcohol, ignited and titrated with *N*/10 acid. This gave 0.0857 gram malic acid or 85.7 per cent. of the amount originally taken. The addition to the filtrate and washings of an equal volume of 95 per cent. alcohol gave an additional precipitate, showing that 75 per cent. alcohol was not strong enough to precipitate all the malic acid. Pure 95 per cent. alcohol precipitated the calcium acetate as a jelly-like mass so that it could not be used without dilution.

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[1] Two cc. of 10 per cent. $Ca(C_2H_3O_2)_2.3H_2O$ contain about the same amount of calcium as 1 cc. of 10 per cent. $CaCl_2$.

As the result of a series of experiments it was found that 85 per cent. alcohol would not precipitate the calcium acetate and, on the other hand, the precipitation of the calcium malate was practically complete. During the course of the experiments it became evident that it was unnecessary to let the precipitate stand over night before filtering, as the filtrate from a half hour's heating on the water-bath remained perfectly clear on standing. This made it possible to complete a determination in an hour's time. Thus 10 cc. of a 1 per cent. malic acid solution, 2 cc. of 10 per cent. calcium acetate, 100 cc. 95 per cent. alcohol, heated for twenty minutes on the water bath or until the precipitate settled, leaving the supernatant liquid clear, filtered and washed with 85 per cent. alcohol (90 cc. of 95 per cent. alcohol + 10 cc. water) incinerated and titrated with $N/10$ hydrochloric acid, required 14.8 cc. $N/10$ acid, equivalent to 0.0992 gram malic acid or 99.2 per cent. of the total. The filtrate and washings gave no appreciable precipitate on the addition of an equal volume of 95 per cent. alcohol.

The determination of malic acid in maple products was next taken up. The addition of alcohol alone to maple sirup gives a precipitate which proved to be calcium malate. The addition of calcium chloride to the filtrate gives an additional precipitate while the addition of calcium acetate to the filtrate from the calcium chloride precipitate, gives a further precipitate, showing the presence of some free acid or of an acid salt. To confirm this, 6.7 grams of maple sirup were diluted with water and titrated directly, using phenolphthalein as indicator—1.4 cc. $N/10$ sodium hydroxide were required, proving an acid reaction. Hence in the experiment cited above—the precipitate with alcohol alone was calcium malate as such—with calcium chloride, the malic acid combined with potassium or other bases and with calcium acetate, the free malic acid. Owing to the unlikelihood of the existence of neutral calcium malate and free malic acid in the same solution, it was deemed probable that they were combined as an acid salt. Accordingly the properties of the pure acid salt were next investigated. The salt $Ca(C_4H_4O_5)_2.6H_2O$ was prepared by saturating hot 10 per cent. nitric acid with neutral calcium malate and allowing to crystallize. An approximately 1 per cent. solution of these crystals was then made up. The average of several closely concordant determinations of the malic acid, by evaporation and ignition of an aliquot portion and titration of the residual lime, also titration of the solution direct, gave 0.065 gram malic acid per 10 cc. of solution.

Ten cc. of this solution, 1 cc. of calcium chloride solution, made up to 20 cc. with water, 60 cc. of 95 per cent. alcohol as outlined in *Bulletin* **105**, *Bureau of Chemistry*, gave no precipitate whatever, so that under these conditions only the malate existing in the maple product as the neutral salt is recovered. Upon the addition of a slight excess of ammonia to the above solution, as recommended on p. 73, *Bulletin* **107**, *Bureau of Chemistry*, warming and allowing to stand over night, and subsequent titration of the ignited precipitate, 0.1052 gram malic acid was found, equivalent to 162 per cent. of that originally present, while the addition of an equal volume of 95 per cent. alcohol to the filtrate gave a further precipitate, showing that, as with the free acid, 75 per cent. alcohol does not completely precipitate the malate.

Next, 10 cc. of the acid malate solution were taken, 2 cc. of 10 per cent. calcium acetate solution, 100 cc. of 95 per cent. alcohol, the solution heated on the water bath, filtered, washed with 85 per cent. alcohol, ignited and titrated, giving 0.064 gram malic acid, equivalent to 98.5 per cent. of that originally present.

Several samples of maple sugars and sirups of known purity were then run through for malic acid, with the following results:

| | | Ash. Per cent. | Malic acid. Per cent. |
|---|---|---|---|
| 1 | Canada Maple Sugar...... | 1.45 | 1.22 |
| 2 | Vermont " " ...... | 0.93 | 0.87 |
| 3 | " " Sirup...... | 0.52 | 0.51 |
| 4 | Ohio " " ...... | 0.53 | 0.52 |
| 5 | Canada " " ...... | 0.79 | 0.68 |

Known amounts of malic acid were then added to these. Following is an example of the results obtained. Five cc. of the 1 per cent. acid calcium malate solution containing 0.0325 gram malic acid equivalent to 0.48 per cent. on a 6.7 grams sample were added to 6.7 grams of sample No. 2 of Vermont maple sugar and 1.33 per cent. malic acid found. Subtracting the 0.48 per cent. added, gives 0.85 per cent. acid in the original sugar which agrees very closely with the 0.87 per cent. found directly.

Pure sucrose was tried with the same reagents. No precipitate formed in a half hour. On standing over night a slight precipitate of calcium carbonate was thrown down, while on longer standing the sucrose started to crystallize. However, if the method is carried out as outlined, no appreciable error can creep in from the reagents, while apparently very concordant results are obtained by its use.

The method finally adopted as the one to secure the most complete precipitation was as follows: 6.7 grams of the sugar or sirup were weighed and dissolved in 5 cc. of water. This quantity was found ample to effect complete solution even of a sugar. Two cc. of a 10 per cent. solution of calcium acetate were then added. Then 100 cc. of 95 per cent. alcohol were stirred in and the solution warmed on the water bath until the precipitate settled readily leaving the supernatant liquid clear. The precipitate was then filtered, washed with 85 per cent. alcohol to freedom from soluble calcium salts, about 75 cc. being required, ignited and an excess of $N/10$ hydrochloric acid added and warmed gently until all carbon dioxide was expelled. It was found that prolonged heating did not drive off any hydrochloric acid, providing the solution was not boiled but was kept at such a temperature that only an occasional bubble was formed. The solution was then cooled and titrated back with $N/10$ sodium hydroxide. One-tenth the number of cc. $N/10$ acid used gives the per cent. of malic acid.

The method is also applicable to fresh and boiled ciders and to cider vinegars.

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[CONTRIBUTIONS FROM THE HAVEMEYER LABORATORIES OF COLUMBIA UNIVERSITY, No. 155.]

# THE EFFECT OF PASTEURIZATION UPON THE DEVELOPMENT OF AMMONIA IN MILK.

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A method for the determination of ammonia in milk and the results of examination of a number of samples, fresh, stale and preserved by means of antiseptics, have been described in previous papers from this laboratory.[1] These determinations as well as the colorimetric tests for ammonia in milk recorded by Trillat and Sauton[2] appeared to warrant a further study of the ammonia content of milk as a measure of protein decomposition and the effect of pasteurization upon the development of ammonia and upon the acid: ammonia ratio. An especial study of the effect of pasteurization was suggested by the well-known bacteriological observation that milk may contain peptonizing or putrefactive bacteria capable of withstanding pasteurization and developing with increased vigor after the destruction of the acid-forming organisms. If this is commonly the case, it would seem that there should be chemical evidences of an increased breaking down of proteins in the pasteurized, over that in the corresponding raw milk.

The method used in the present investigation was the same as has been described by Berg and Sherman (*loc. cit.*) and its accuracy was again verified by blank tests and check determinations with known amounts of ammonia salts. The essential feature of this method, which is an adaptation of that of Boussingault[3] and Shaffer,[4] is that the milk is diluted with an equal volume of methyl alcohol, made alkaline with sodium carbonate and distilled under diminished pressure. Under these

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[1] Berg and Sherman, THIS JOURNAL, **27**, 124 (1905); Sherman, Berg, Cohen and Whitman, *J. Biol. Chem.*, **3**, 171 (1907).

[2] *Bull. soc. chim.*, **33**, 719 (1905).

[3] *Ann. chim. phys.*, **29**, 472 (1850); *J. prakt. Chem.*, **51**, 281 (1850).

[4] *Am. J. Physiol.*, **8**, 330 (1903).