

In the application of the above formula to obtain the velocities of sound in the two non-metals cited the value of the constant L is to be multiplied by 10.

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¹ Sen, B. N., *Gazetta*, 1938, **10**, (68), 662.

² —, *Proc. National Academy of Science, India*, 1937, **8**, 1, 6.

³ *International Critical Tables*, **6**, 465.

⁴ Sen, B. N., *Journ. Ind. Chem. Soc.*, 1934, **11**, (4), 243.

⁵ —, *Gazetta*, 1938, **10**, (68), 656.

THE MILK CLOTTING ENZYME OF *WITHANIA COAGULANS*

THE fruit of *Withania coagulans* contains an active rennet which can be obtained in highly concentrated form by the following procedure: The partially dried fruits are ground up with water, the extract filtered through paper pulp and the clear solution treated with ammonium sulphate. The precipitate formed at 25 per cent. saturation is discarded as it contains very little activity. The material that separates on further addition of ammonium sulphate to 65 per cent. saturation contains the whole of the enzyme. The precipitate is separated by centrifuging, redissolved in water, and the solution after being dialysed free from ammonium sulphate, is filtered through paper pulp. Ten volumes of acetone are now added, the precipitate is centrifuged, washed with small quantities of acetone and dried in the desiccator. 100 g. fruit pulp usually yield about 3 g. of enzyme. The material thus obtained is a brownish white powder which has a milk coagulating action nearly 30 times that of the original fruit pulp, 0.125 g. of powder being capable of bringing about the coagulation of 1 litre of fresh milk at 30° in 30 minutes. The preparation is quite stable and retains its activity unimpaired on keeping at room temperature for weeks.

For determination of activity comparison was made with a standard pepsin solution prepared according to Rona¹ (1931) the substrate being either freshly boiled milk (Michaelis and Rothstein)² or milk powder (Rona and Gabbe).³ The optimum temperature for the action of enzyme is 48°. Three minutes at 90° completely destroys it, the destruction being 40 per cent. at 70° and 75 per cent. at 80°. The main properties of the enzyme from *Withania coagulans* as compared to those of other well-known milk clotting enzymes are given in the following table.

	Enzyme from <i>Withania coagulans</i>	Papain	Pepsin
ACTIVITY (Quantity of enzyme for clotting 1 lit. of milk in 30 min.)	125 mg.	31 mg.	3.2 mg.
Optimum Temperature	48°	87°	37°
Proteolytic action	—	+	+

It will be seen that the preparation from *Withania coagulans* is only about 1/4 as active as papain and 1/40 as active as pepsin. In practical cheese making however it is doubtful if papain can be utilised as a substitute for gastric rennet on account of the bitter flavour it imparts to the clot even in minute concentration. The texture of the clot formed is dependent on the time taken which is in turn determined by the quantity of enzyme and the temperature. A firm compact clot is obtained when at the optimum temperature of 48° sufficient enzyme is added to give a clot in about 20 minutes.

A finding of considerable theoretical importance is the observation that the *Withania coagulans* enzyme has no proteolytic action, no increase in amino nitrogen being observed when it is allowed to act for a week on gelatin solution at various pH's. On account of the difficulty of separating gastric rennet from pepsin the individuality of the former has often been questioned. Further in discussions on the mechanism of clot formation a proteolytic fission

of the casein molecule prior to coagulation has frequently been postulated (cf. Oppenheimer).⁴ In the enzyme now obtained from *Withania coagulans* we have for the first time a preparation which is entirely devoid of proteolytic activity and which therefore provides clear proof of the independence of the process of coagulation to hydrolytic cleavage of casein.

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¹ Rona, *Praktikum der physiologischen chemie*, 1931, **1**, 276.

² Michaelis and Rothstein, *Biochem. Zeit.*, 1920, **105**, 60.

³ Rona and Gabbe, *Ibid.*, 1922, **134**, 39.

⁴ Oppenheimer, *Die Fermente*, 1926, **1**, 978.

CATALYSIS BY ASCORBIC ACID

DURING the course of our work on the role of ascorbic acid in physiological processes and the cause of its stability in plant and animal tissues we have found that it catalyses the reduction of silver chloride by sodium sulphite.

The experiments were conducted in brown bottles and silver chloride was formed *in situ* by adding to each bottle 10 ml. of 0.1N silver nitrate and 10 ml. of 0.1N potassium chloride solution. The requisite volumes of sodium sulphite solution and ascorbic acid solution were then added, followed by enough distilled water to make up the total volume to 50 ml. After three to three and half hours, the contents of each bottle were poured through a filter (Whatman No. 42, for fine precipitates). The residue on the filter was carefully washed until free from the soluble salts. The funnel with the filter is then put over a 250 ml. volumetric flask and the residue on the filter treated with 1:1 dilute analytical nitric acid. The corresponding brown bottle was also treated similar-

ly and the liquid poured on the filter. The treatment is repeated three times to ensure complete solution of any metallic silver formed by reduction. It is well known that silver chloride does not dissolve in 1:1 nitric acid. The filtrate in the 250 ml. flask was made up to the mark, and the amount of silver in an aliquot portion estimated volumetrically by titration with standard potassium thiocyanate solution, using ferric alum as the indicator.

Under these experimental conditions we have found that sodium sulphite does not reduce silver chloride, while ascorbic acid does so readily. Further we made the interesting observation that in the presence of sodium sulphite a given amount of ascorbic acid produces a much larger reduction of the silver halide than when it is alone.

TABLE I
5 Milligrammes of ascorbic acid

Volume of sodium sulphite solution 0.025 Molar	Amount of AgCl in milligrammes Ag	Milligrammes Ag obtained by reduction in 3½ hours
0	107.9	2.88
5 ml.	107.9	6.26
10 ml.	107.9	9.04
15 ml.	107.9	9.71

The results indicate that ascorbic acid induces the reduction of silver chloride by sodium sulphite. The following table shows the influence of the concentration of the inductor, namely ascorbic acid on the rate of reduction, keeping the concentration of sodium sulphite at a constant but fairly high value.

TABLE II
Concentration of sodium sulphite 0.05 Molar

Amount of ascorbic acid	Amount of AgCl in milligrammes Ag	Milligrammes Ag obtained by reduction in 3 hours
5 milligrammes	107.9	7.08
10 „	107.9	12.18
15 „	107.9	17.00