LVIII. THE BEHAVIOUR OF PECTIN TOWARDS ALKALIS AND PECTASE.

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PECTIN is a plant product which plays a part of considerable importance in the jam and fruit-preserving industries, and problems connected with it also arise in the making of wine, cider, perry and other fermented beverages made from fruit juices. It is present in fruits and also in roots, such as carrot, turnip etc., in which parenchymatous tissue is largely developed. A solution of pectin in presence of suitable quantities of sugar and an acid readily forms a gel, and it is to this property that the "setting" of jams and jellies is due. It may be prepared by the addition of alcohol to an aqueous extract of the respective fruit, when the pectin separates as a voluminous, gelatinous precipitate. Although the product so obtained cannot be considered in the strict, chemical sense as a pure compound no doubt can be entertained that it is, essentially, an individual substance. It cannot be crystallised, and the only methods of purification which have, so far, been found practicable are treatment in aqueous solution with animal charcoal and reprecipitation with alcohol.

The literature on the subject of pectin is voluminous, but a perusal of it reveals a great deal of confusion, and contradictory statements are frequent.

The present communication embodies the results of some preliminary work on pectin, and deals with the action of alkalis and of the enzyme, pectase, on this substance. It has long been known that pectin, on treatment with an alkali, yielded pectic acid, a substance which is insoluble in water in presence of an appreciable amount of an electrolyte. More recently Th. von Fellenberg [1914] has shown that the product obtained on distilling pectin with aqueous alkali contains methyl alcohol. Although he never made any thorough examination of the distillate he concluded, from indirect evidence obtained by several methods, that methyl alcohol was the only volatile product formed. To the present author, however, this conclusion did not seem to be justified, and investigation has proved this to be the case. Lippmann [1920] speaks of it as a well-known view that pectin is a methyl ester of "pectinic" acid, and attributes the presence of methyl alcohol in rum to the decomposition of pectin derived from the sugar cane.

No definite information appears, so far, to have been obtained regarding the action of the enzyme, pectase, on pectin beyond the fact that it causes a solution of the latter to set to a stiff gel, and that this change occurs more readily in the presence of chalk. The production of the gel has been considered to be due to the formation of calcium pectate.

The present author finds that the actions of cold, dilute alkali and of pectase on pectin are essentially identical. In both cases a salt of pectic acid is formed and not only methyl alcohol, but also acetone, is eliminated. In this connection it is worthy of record that both methyl alcohol and acetone have now been found to be present in cider, being doubtless derived from the pectin, which becomes destroyed during the fermentation of the apple juice.

Both the acetone and the methyl alcohol are eliminated with great facility from pectin, a small quantity of $N/10$ alkali at ordinary temperatures being sufficient to set free both compounds. This would suggest that both of them were present as ester groupings, the acetone occurring as an ester of its enolic modification, isopropenyl alcohol, $\text{CH}_3\text{C}(\text{OH}) : \text{CH}_2$. It appears likely, therefore, that pectin is the dimethylisopropenyl ester of pectic acid.

EXPERIMENTAL.

The pectin employed in this investigation, except where otherwise stated, was obtained from apples. Dried "pomace" (the "press cake" from the cider press) was heated to 100° with water and the liquid subsequently expressed. The pectin was then precipitated by the addition of alcohol to the dark-coloured solution, collected, pressed free from mother-liquor, and purified by solution in water and re-precipitation by alcohol. As thus obtained the pectin formed a brownish, voluminous, jelly-like precipitate, which could be deprived of a large volume of occluded mother-liquor by careful pressing in a cloth. Further purification was effected by treatment with animal charcoal in dilute aqueous solution. The first treatment with animal charcoal caused an appreciable diminution of the amount of material in solution, but subsequent treatments resulted in the absorption of only small quantities of the pectin, thus indicating that the initial loss was due essentially to the removal of impurities. On precipitating, the pectin after this purification separated as an almost colourless, jelly-like precipitate which, when dried, formed a greyish solid which could be reduced to a nearly white powder. The yield was nearly 5 % of the weight of the dried pomace employed.

The action of alkali on pectin.

All samples of pectin were found to possess a slightly acid reaction and the purified material (dried in a vacuum) when titrated, neutralised immediately in the cold 1.05 % of its weight of sodium hydroxide. On keeping, however, in aqueous solution at ordinary temperature in presence of an excess of the alkali a further amount of the latter equivalent to 12.35 % of the weight of

the pectin employed was taken up, the reaction being practically complete in half-an-hour, only a trace more alkali being neutralised on keeping for a further 18 hours. On then adding an excess of acid to the solution pectic acid was precipitated. When pectin was heated to about 80° with excess of $N/10$ alkali, however, much more of the latter was neutralised, considerable darkening occurred, and no definite end-point to the reaction could be ascertained. Moreover, on acidifying the alkaline mixture after heating for two hours no pectic acid was precipitated.

It has been recorded by von Fellenberg [1914] that pectin yields methyl alcohol when distilled with an excess of aqueous alkali, and this reaction was therefore investigated. A considerable quantity of pectin, in aqueous solution, was treated with an excess of sodium hydroxide and the mixture distilled in a current of steam for some time. It was at once evident that the distillate contained something other than methyl alcohol since it readily yielded iodoform on treatment with iodine and an alkali. The entire distillate¹ was therefore submitted to a systematic process of fractional distillation, employing a Young's rod and disc column, when it was separated into water and a fraction boiling below 70° . The latter, after drying over quick lime, was re-fractionated several times, again employing an efficient column, when it passed over from $56-66^{\circ}$ and was finally collected in eight fractions, all of which were analysed. The fraction boiling at 66° consisted of methyl alcohol (found: $C = 37.5$, $H = 12.6\%$), whilst that boiling at $56-58^{\circ}$ evidently consisted chiefly of acetone. It has a strong odour of the latter, gave $C = 58.5$, $H = 10.8\%$, and readily yielded acetonesemicarbazone, melting at 186° . The regular change in the composition of the intermediate fractions, as shown by analysis, clearly indicated that no third substance was present. The proportion of acetone to methyl alcohol produced appeared to be about one part of the former to two of the latter.

Pectin was then treated in cold, aqueous solution with 13.4% of its weight of sodium hydroxide (the amount taken up by pectin in the cold), the mixture kept for 30 minutes, then made slightly acid, and distilled as before. It was then found that both acetone and methyl alcohol were formed, and to the same extent and in the same proportion as when the distillation had been conducted in the presence of a considerable excess of alkali. Moreover, when an amount of alkali sufficient to react with only half the pectin taken was employed both acetone and methyl alcohol were obtained, and apparently in the same relative proportions as when an excess of alkali had been used.

The action of pectase on pectin.

A quantity (13 g.) of pectin was dissolved in 2 litres of water, a few grams of precipitated calcium carbonate added, and then 40 cc. of pectase solution introduced. This was prepared by pounding fresh clover in a mortar with a

¹ The original distillate was always alkaline owing to the presence of a trace of ammonia, doubtless derived from a small amount of protein material present as an impurity in the pectin. It was neutralised before fractionation.

little water and then expressing the liquid from the resulting mass. On keeping over night the whole mixture set to a jelly. The latter was broken by vigorous shaking and distilled in a current of steam. On fractionating the distillate it was found to contain acetone and methyl alcohol to the same extent as if the pectin had been distilled with alkali.

The contents of the distillation flask were then filtered, and the filtrate concentrated to a small bulk under diminished pressure and precipitated with alcohol. A small quantity of an amorphous solid was thus obtained, which was not unchanged pectin, but probably represented impurities in the original sample of pectin used, whilst the filtrate from this amorphous solid contained only a very small amount of material, largely dextrose, which was doubtless derived from the clover extract which had been added. Pectin, when treated with pectase in the presence of chalk¹, therefore appears to yield only acetone, methyl alcohol and the gelatinous solid which consisted of calcium pectate, as shown below. The action of the enzyme, pectase, on pectin is therefore precisely analogous to that of sodium hydroxide, since the latter has been shown to convert pectin into acetone, methyl alcohol, and sodium pectate.

The solid particles of jelly which had been separated by filtration from the liquid in the distillation flask were macerated in the cold with dilute hydrochloric acid, the mixture filtered and the solid washed until free from calcium. After treatment in solution as sodium salt with animal charcoal the acid was reprecipitated, washed, dried in a vacuum and analysed. Found: C = 39.2, H = 5.1, NaOH neutralised = 20.86 %. Pectic acid prepared by the action of sodium hydroxide in the cold on pectin gave C = 39.4, H = 5.1, NaOH neutralised = 20.90 %.

Pectin from other sources.

In order that the pectin from plants belonging to different Natural Orders might be compared with the product from apples, carrots, turnips and mangold wurzels were investigated. It was then found that the pectin from each of these sources behaved like that from apples when treated with pectase or with alkali, yielding in each case both acetone and methyl alcohol.

The product obtained from mangold wurzels, however, was obviously much less pure than apple pectin, and yielded quite an appreciable amount of ammonia when distilled with alkali.

REFERENCES.

- von Fellenberg (1914). *Chem. Zentr.* **11**, 942.
Lippmann (1920). *Biochem. Zeitsch.* **106**, 236.

¹ Chalk was added since the presence of free acid retards the action of pectase.