STUDIES IN THE PREPARATION, PROPERTIES AND ASSAY OF COMMERCIAL PAPAIN

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SUMMARY

STUDIES of a fundamental and practical nature on the latex of the papaya fruit have shown that :—

(1) Papaya latex coagulates rapidly on stirring in a flat dish as a result of physico-chemical changes brought about by atmospheric oxidation.
(2) The oxidation of papaya latex is promoted by oxidizing enzymes belonging to the class called peroxidases.
(3) Common salt is quite suitable as a coagulating and protective agent for papaya latex.
(4) Commercial papain of good quality can be prepared from papaya latex by sun-drying or oven-drying for 6 to 8 hours between 50° C and 55° C or by drying to the consistency of a fairly hard paste of composition about 20 per cent. added common salt, 45 per cent. papaya latex solids and 35 per cent. moisture.
(5) Papaya latex should be dried to a moisture content below 10 per cent. unless common salt is used as a protective agent. Samples having a moisture content above 10 per cent. lose their activity very rapidly and undergo a change in colour from pale cream to dark brown within a period of about one year.
(6) A simple modification of the gelatine viscosity method can be used for comparing the proteolytic activities of samples of commercial papain.
(7) Good quality commercial papain (not treated with common salt) should have a pale cream colour, a pungent but not unpleasant odour, a moisture content not exceeding 8 per cent., a minimum nitrogen content of 11·5 per cent. on a moisture free basis, and a proteolytic activity not less than 70 per cent. of a freshly prepared sample.

INTRODUCTION

Commercial papain is prepared by sun-drying, oven-drying or vacuum-drying the latex extracted by lancing the mature green fruit of the papaya tree (*Carica papaya*).

The cultivation of the papaya tree, the method of lancing the fruits and extracting the latex and drying it, have been described in a previous number of this journal (1). This data has been very valuable in many respects. However, as a result of recent advances in our knowledge of the properties of the latex, further investigation appeared desirable in order to improve the local methods of production of commercial papain.

Commercial papain is widely used as a raw material in industry for pharmaceutical preparations, for clearing fruit juices and fermented liquors, for tenderizing meat and for preshrinking and improving the handle of wool (2).

Papaya latex is known to contain at least two proteolytic enzymes called papain and chymopapain (2). These enzymes lose their activity as a reslt of atmospheric oxidation promoted by certain substances present in the latex. The nature of these substances will be discussed at a later stage in this paper.

The inactivation of the proteolytic enzymes in commercial papain can be reversed by means of certain reducing agents like hydrogen sulphide, hydrogen cyanide, sulphides and sulphites. After prolonged exposure to oxidizing influences, however, the material is no longer capable of reactivation (3). Therefore, commercial papain does not keep indefinitely and it is necessary that its proteolytic activity should be determined, in order to ascertain its quality.

METHODS OF ASSAY

The methods of determining the proteolytic activity of commercial papain described in a previous number of this journal (1), have been abandoned by modern workers as they were found to be cumbersome and not accurate enough. The tentative casein digestion method of the A.O.A.C. (4), was tried out and found to involve the use of as much as 200 cc of 95 per cent. ethyl alcohol for every titration. Moreover, the method itself was rather cumbersome, though accurate enough for ordinary purposes. Eventually the modified milk clotting method of Balls and Hoover (5), was used with satisfactory results.

It was brought to the writer's notice that a simple and quick method of determining the proteolytic activities of samples of commercial papain would be useful to the producer. The gelatine viscosity method (6), was therefore modified and a simple apparatus in the form of a capillary-pipette of about 25 cc. capacity devised. The pipette was 45 cm. long and had a capillary tube 15 cm. long of 1 mm. bore, attached to the end. The time of flow of a 5 per cent. aqueous solution of gelatine at room temperature, at about 30°C, from a point marked at the top stem of the pipette to a point marked at the bottom stem should be about sixty seconds. The procedure adopted was as follows :—

A 0·1 per cent. aqueous solution of the commercial papain was prepared by grinding the product to a paste with water and then diluting it. 1 cc. of this solution was then added to 30 cc. of a 5 per cent. aqueous solution of gelatine prepared by warming the gelatine gently with water until it was completely dissolved and cooling it to room temperature. The two solutions were well mixed and allowed to remain at room temperature for 15 minutes. The time taken for the gelatine solution to flow from one point in the capillary-pipette to the other as described above was noted by means of a stop-watch before

and after treatment with the commercial papain solution. The time of flow (t) in seconds is given by the formula :—

$t = K\frac{v}{d}$, where (v) is the viscosity coefficient, (K) a constant for the particular pipette and (d) the density of the gelatine solution. Now, if t_1 and t_2 are the times of flow before and after treatment with the commercial papain solution, repectively, then the proteolytic activity (A) is given by the relationship :— $A = \frac{t_1 - t_2}{t_1}$. The change in density due to the addition of 1 cc. of the commercial papain solution to 30 cc. of the gelatine solution was negligible.

It should be noted that by this method, it is only possible to state whether a particular sample is inferior or superior to another, as there is no simple relationship between activity and enzyme concentration (6).

EXPERIMENTAL

The investigations on the properties of papaya latex and its behaviour when exposed to air indicated that the coagulation of the latex was a physico-chemical change brought about by atmospheric oxidation. This was accompanied by a fall in pH from 6·2 to 5·8 and was probably due to the formation of acidic substances.

The fresh latex had a moisture content of about 80 per cent. It took about 4 to 6 hours to coagulate on standing ; but rapid coagulation occurred in about 10 minutes when it was poured into a flat dish to form a thin layer, and then stirred vigorously.

Papaya latex was observed to keep fresh for about 4 hours without appreciable deterioration in activity. On keeping for longer periods, however, decomposition set in with the development of an offensive odour and accompanied by a rise in pH to 7.2 in about 24 hours. This decomposed product gradually darkened to a brown colour on drying. This darkening was probably due to the oxidation of a chromogenic substance present in the latex, as in the case of apples (7). The darkening of papaya latex was further enhanced by the use of iron utensils. Iron appears to catalyse the above reaction. The presence of oxidizing enzymes belonging to the class called peroxidases in the latex, was demonstrated by the benzidine colour reaction, in the presence of hydrogen peroxide (8). These enzymes are thermostable and promote oxidative changes which reduce the proteolytic activity of papaya latex during drying and storage (5).

It was observed by Balls *et al.* (5), that common salt had a remarkable coagulating effect on the latex. The writer observed that the fresh latex required about 3 per cent. by weight of common salt to coagulate it. Moreover, common salt was reported to have a protective effect on the proteolytic enzymes in the latex, provided the treated latex was not completely dried A paste prepared by partially drying the latex treated with common salt.

to give a final composition of 35 per cent. papaya latex solids, 20 per cent. common salt and 45 per cent. moisture, was found to have undergone no appreciable loss of activity on exposure to air at about 30°C. for 14 days. It did not lose activity to any appreciable extent for as long as 50 days and less than 10 per cent. in 190 days, when kept in airtight vessels. This method was investigated by the writer and it was confirmed that a minimum concentration of 20 per cent. common salt in the final product was necessary to produce a paste of satisfactory consistency. A sample of paste was prepared by adding 10 per cent. of common salt by weight to the latex and drying the mixture in the sun to a stage when the material became thick and plastic. This product was found to contain 20 per cent. common salt, 45 per cent. papaya latex solids and 35 per cent. moisture.

Papaya latex after coagulation can be converted into thin uniform worms by passing it through a colander, string-hopper squeezer or potato-squeezer, having tiny holes. These utensils could be made of aluminium, brass or stainless-steel. If iron is used, it should be heavily tinned. The material was distributed uniformly in thin layers on drying trays covered with muslin cloth. The material was then dried in the sun or in a well ventilated drier between 50°C. and 55°C., for about 6 to 8 hours, to give a pale cream product with a moisture content below 8 per cent. The dried product should be quite crisp and crumble easily to a powder when crushed. Products not treated with common salt and having a moisture content above 10 per cent. were found to deteriorate very rapidly even on storage in airtight bottles. They lost proteolytic activity almost completely with the development of an offensive odour and changed in colour from pale cream to dark brown within an year. It was observed that good quality commercial papain (not treated with common salt) generally had a moisture content of 5 per cent. to 8 per cent. and a nitrogen content of 11·5 per cent. to 11·8 per cent. on a moisture-free basis. It had a pale cream colour and a pungent but not unpleasant odour. Commercial papain in the form of a half dried paste containing 20 per cent. common salt was observed to have a pale cream colour and to retain the pleasant odour of fresh papaya latex for a longer period than the former.

METHODS OF PRODUCTION

The above investigations indicate that commercial papain of good quality can be produced under local conditions by the following methods :—

(1) The latex is extracted by lancing the mature green fruits lightly on the surface by means of a piece of glass with a sharp edge, a stainless steel knife or a bamboo splinter with a sharp edge. This operation is done in the mornings before 10 A.M.

(2) The latex is collected in glass or porcelain vessels. Coconut-shells or the leaf-sheaths of arecanut palms could also be used. The latex is pooled and then poured into flat enamel or earthen-ware vessels and stirred vigorously by means of a wooden spoon till it coagulates.

(3) The coagulated latex is immediately passed through a colander, string-hopper squeezer or a potato-squeezer, made of aluminium, brass or stainless-steel. If iron is used, it should be

heavily tinned. The material is exuded as thin uniform worms and is evenly distributed in thin layers on drying trays covered with muslin cloth.

(4) The material is dried in the sun or in a well ventilated drier at 50°C. to 55°C., till it becomes crisp. The product takes about 6 to 8 hours to dry to a moisture content below 8 per cent.

(5) If producers wish to use common salt as a protective agent, a half dried paste is prepared by adding 10 per cent. by weight of common salt to the latex. The subsequent process is as described in para. 3. The material is dried in the sun or in the drier at 50°C. to 55°C., until it becomes thick and plastic. The product takes about 2 to 3 hours to dry to this consistency.

(6) Commercial papain should be packed immediately after drying in tins free from rust and sealed airtight. It is preferable to evacuate the air from the tins and seal them to ensure good keeping quality. The tins should be kept in a cool place preferably under cold storage.

(7) The product, soon after packing in tins, should be transported with as little delay as possible to the central clearing house.

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