

SOME ASPECTS OF ENHANCEMENT OF PROTEOLYTIC ACTIVITY OF PAPAIN

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Abstract

Sun drying of papain, the most commonly used method, in Sri Lanka was found to yield papain of inferior quality. Both oven drying and vacuum drying were found to be superior to sun drying. Vacuum drying yielded the highest proteolytic activity. Oven drying at 50 °C gave best results. Proteolytic activity of crude papain can be enhanced by the addition of sodium metabisulphite (SMBS) or thymol to raw papaya milk prior to drying. Combined addition of both sodium metabisulphite and thymol followed by vacuum drying yielded unpurified papain with the highest proteolytic activity. A new processing procedure tested gave satisfactory results.

1. Introduction

The quality of papain (dried papaya latex of *Carica papaya L*) is dependent on proteolytic activity which in turn determines its commercial value. Method of preparation of papain is known to have a significant effect on the quality of final product. Optimum tapping methods have been reported by Madrigal *et al* (1980). The storage and drying characteristics of papaya latex has been investigated by Ortiz *et al* (1980). It is known that both sun drying and oven drying are used by papain producers resulting in white (Grade No 1) and brownish papain (Grade No 2) respectively. Ortiz *et al* (1980) have mainly investigated the effect of oven drying at different temperatures on proteolytic activity. Similar studies have been carried out by Hinkel (1951). Preliminary studies carried out by us showed that method of drying has a profound effect on the proteolytic activity of resulting papain. This aspect was further investigated with a view to enhancing the quality of papain. The addition of chemical activators was also investigated.

Several methods have been reported for extraction and purification of papain from papaya latex (Arnon, 1970, Kimmel & Smith, 1954). However, these methods require advanced laboratory facilities, limiting their use at

non-laboratory level. A comparatively simple purification procedure therefore, was attempted to upgrade the quality of papain and the results are reported.

2. Experimental

Latex collection:

Samples of papaya latex were collected from about 10 healthy trees of *Carica papaya* found in the University garden. Half mature green fruits were tapped by making several vertical incisions and the latex was collected in glass petri dishes. Fluid latex was then allowed to autocoagulate, unless otherwise mentioned; autocoagulation occurred within 30 min of collection. Latex from different trees were pooled and mixed while they were still in a fluid state.

Pre-drying treatments:

The addition of chemical activators (eg sodium metabisulphite, 0.5% w/w; thymol, 0.2% w/w) was done prior to autocoagulation on a fresh weight basis. Papain after drying was powdered in order to ensure complete mixing of the activators with the enzyme.

Purification of papain:

The following modified procedure based on a method used by Benren (1987) was adopted for purification of papain.

Papaya latex was collected from apparently healthy trees into clean containers and was kept away from light and processed within 24 h.

Sodium bisulphite was added to papaya latex to a final concentration of 0.15% by weight. This was taken into a cloth bag and knead repeatedly with hands, using gloves, to make the latex peptized.

Peptized latex was then collected into a centrifuge tube and centrifuged at 3000 - 4000g for 15 min.

Supernatant was collected. The solid fraction was squeezed by using a cloth bag and the liquid resulting was combined with the above supernatant.

The pH of the combined liquids was adjusted to 4.5 by using 0.1N HCl and the liquid incubated in a water bath for 40 min at 45°C.

Kieselghur was added to the liquid, 10% by weight and filtered under reduced pressure.

The pH of the filtrate was then adjusted to 7.2 with dil NaOH. Filtrate was then concentrated in a laboratory thin film evaporator at 40°C.

Concentrated sample was then lyophilized at -10°C or dried in desiccator at room temperature.

Proteolytic activity assay:

The assay of proteolytic activity was based on the method of Arnon (1970). Papain samples were dissolved in (0.04mg/ml) in 0.05M phosphate buffer, pH 6.0, containing EDTA 38mmol/l and cysteine 34 mmol/l. Incubation medium consisted of 5.0 ml casein solution (1% in 0.05M phosphate buffer pH 6.0), 1.5 ml of 0.05M phosphate buffer, pH 6.0 containing EDTA 38 mmol/l & cysteine 34mmol/l and 0.05ml of papain. Incubation period was 60min at 37°C and the reaction was stopped by the addition of 3.0 ml of 30% TCA. It was then kept for 30 min at 37°C and filtered through Whatman No 2 filter paper.

The absorbance of the filtrate was measured at 280 nm using a spectrophotometer. These absorbances were referred to the standard absorbance of L-tyrosine under the same conditions. Activities were expressed as international units (IU), ie μmol s of tyrosine liberated min mg of sample. All samples were analysed in duplicate, sometimes in triplicate.

Protein content of purified papain samples was determined by the method of Updegraff (1971) using Beisenherz reagent.

3. Results

Effect fo drying temperature on proteolytic activity:

The effect of drying temperature of papain on proteolytic activity was studied and it was found that drying of auto-coagulated papaya latex around 50°C yields papain with the highest proteolytic activity (Fig 1).

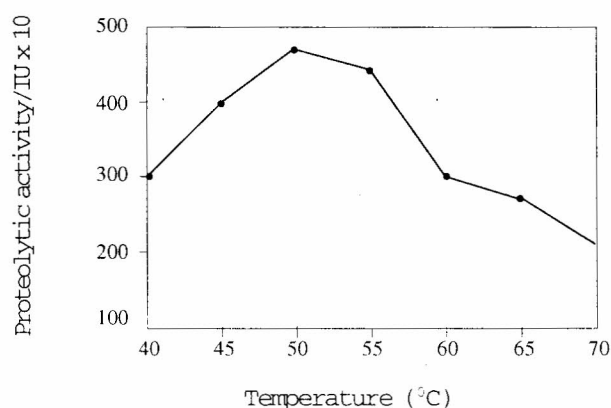


Fig. 1 Effect of drying temperature on proteolytic activity of papain

Effect of drying method on proteolytic activity

Effect of drying method on proteolytic activity of papain was studied. Drying in the sun in open vessels, drying in the oven at 50°C and vacuum drying at room temperature were investigated. Of them, vacuum drying was found to be the best method of drying for papain followed by oven drying at 50°C. Sun drying gave papain with the lowest activity and the resulting papain was brownish in colour probably due to oxidation of phenolic compounds (Tables 1 & 2). Vacuum drying and oven drying yielded white papain.

Table 1 Effect of method of drying on proteolytic activity of crude papain

Method	Proteolytic activity (IU)
Sun	0.207 ± 0.11
Oven*	0.262 ± 0.04

* at 55°C; (p=0.52)

Table 2 Effect of vacuum drying on proteolytic activity of crude papain

Method	Proteolytic activity (IU)
Sun	0.292 ± 0.02
Oven*	0.320 ± 0.05
Vacuum**	0.350 ± 0.03

* at 55°C, ** at 29°C

Effect of addition of chemical activators

The addition of potassium metabisulphite (PMBS), 0.5%w/w; sodium metabisulphite (SMBS), 0.5% w/w and thymol, 0.2% w/w, as activators was investigated. SMBS was found to enhance the activity of papain (Table 3). Although PMBS too enhanced the proteolytic activity slightly compared to oven dried papain (control); the increase was considerably less than that of SMBS. However, the addition of thymol with PMBS was found to enhance the proteolytic activity but still less than that of SMBS added alone (Table 3). None of these increases with SMBS (p = 0.24, PMBS (p=0.80), PMBS + thymol (p=0.54) was statistically significant.

Table 3 Effect of addition of sodium metabisulphite and potassium metabisulphite on proteolytic activity of crude papain

Treatment	Proteolytic activity (IU)
Control	0.158 ± 0.09
Papain + SMBS	0.235 ± 0.07
Papain + SMBS	0.174 ± 0.09
Papain + SMBS + Thymol	0.201 ± 0.10

(PMBS, potassium metabisulphite; SMBS, sodium metabisulphite; samples oven dried at 55°C)

The effect of addition of SMBS and the effect of drying method was also compared. Vacuum drying of SMBS-added papain gave papain with the highest activity (Table 4)

Table 4 Effect of addition of sodium metabisulphite and the method of drying on proteolytic activity

method of drying	Proteolytic activity (IU)	
	without Smbs	With SMBS
Sun	0.172 ± 0.012	0.180 ± 0.015 (P=0.18)
Oven	0.162 ± 0.005	0.190 ± 0.022 (P=0.24)
Vacuum	0.208 ± 0.106	0.210 ± 0.012 (P=0.84)

(SMBS, sodium metabisulphite)

The effect of addition of SMBS and SMBS+thymol was also studied in relation to drying method. It was observed that the addition of thymol with SMBS further enhances the activity of papain compared to when SMBS is added alone. Papain prepared by addition of SMBS+thymol and vacuum dried, possessed the highest proteolytic activity (Table 5).

Table 5 Effect of addition of sodium metabisulphite and thymol on activity of crude papain

Method of drying	Proteolytic activity (IU)	
	With SMBS	With SMBS + thymol
Vacuum	0.405	0.436
Oven	0.385	0.402
Sun	0.246	0.327

(mean of two determinations)

Purification of papain

Purification of papain by the method based on that of Benren (1987) gave quite satisfactory results with a recovery of 81% (Table 5). Since the final step of the method of Benren (1987) requires the use of a freeze drier, desiccator drying was also attempted as a measure of simplification, in terms of equipment requirement. However, lyophilization was found to be superior to desiccator drying with regard to proteolytic activity of purified papain. (Table 6)

Table 6 Proteolytic activity after purification of crude papain

Sample	Specific activity (IU)/mg protein
Purified and lyophilized	2.49
Purified and desiccator dried	1.97
Control (unpurified)	0.65

(Mean of two determinations)

4. Discussion

Papain is a proteolytic enzyme of plant origin with a wide range of industrial applications. Since papain is usually marketed in crude form (ie dried papaya latex) variability in proteolytic activity is known to be high, (range 0.08 IU-0.32 IU, mean 0.19 IU, S.D. =0.105) showing the importance of paying attention to various factors that would enhance and retain its activity.

The results of this study show that sun drying of papaya latex is not the best method of drying for preparation of papain. It is well known that sun drying is the most commonly used method of drying by many a papain producers and it yields a brownish product with comparatively low proteolytic activity. This was further confirmed when several samples purchased from the local market were tested for proteolytic activity. Both oven drying and vacuum drying were found to be superior to sun drying resulting in a whitish product with a higher proteolytic activity and of the two methods, vacuum drying was found to be the best (Table 2). This could be attributed to reduced oxidation under vacuum conditions, particularly of thiols which are known to act as activators of papain. As far as the drying temperature was concerned, drying at 50°C was found to give the highest activity. This is in accordance with the finding of Alberto *et al* (1980). Apparently, drying temperature is not given due consideration by the local papain producers.

Quality of papain could be improved considerably by encouraging the producers to use optimum conditions of manufacture particularly with regard to method of drying.

It was observed in this study that the proteolytic activity of papain could be enhanced by the addition of sodium metabisulphite (SMBS) and thymol either singly or in combination, the latter being the best. This is complementary to the finding of Krishnamoorthy *et al.* (1960) who reported an enhanced activity due to the addition of thymol and potassium metabisulphite (PMBS). However, in the present study enhancement due to PMBS was quite low compared to that of Krishnamoorthy *et al.* (1960). The activation due to SMBS, on the other hand, was relatively higher. The actual mechanism of activation of papain by thymol is not known. Papain with such enhanced activity due to addition of chemical activators, may be of use in certain applications such as deproteinization of *Hevea* latex by using papain. However, it may not be acceptable for pharmaceutical applications which require highly purified material.

Purification of crude papain was one of the objectives of the present study. Purification procedures for papain have already been reported (Arnon, 1970) but the technological inputs are high and may not be attractive to small scale producers of papain who have limited facilities. In this situation we attempted to develop a method for purification of papain.

The method of purification investigated was based on a procedure used by Benren (1987) in China and yielded a purified product as anticipated in our preliminary studies (Table 6). An occasional loss or reduction in proteolytic activity (specific activity) was observed in purified papain. This infact was found to occur at the peptizing step where the residue appear to retain some activity, leading to purified papain with reduced activity. This could be overcome by washing the residue 2 or 3 times with 0.1M Na_2HPO_4 and NaHSO_3 and combining the washings with the peptized papaya latex (Benren, 1994). However, this step has not been tried out yet in our laboratory. Although this method is comparatively less complicated, the simplification appears to be still insufficient to be introduced at village level. An alternative, would be to set up Group Processing Centres, where the trained personnel could process the papaw milk at a central location, into a high quality product, even with the presently available methods for purification such as the one tested in this study.

In conclusion, it is clear that the proteolytic activity of papain can be enhanced by employing modified preparative methods. Proteolytic activity can be further enhanced by the addition of chemical activators such as sodium meta bisulphite and thymol. Papain with such enhanced activity

should pose no problems in industrial applications such as enzyme deproteinization of *HEVEA* latex (Yapa & Yapa, 1984). Purification without chemical additives will be required for pharmaceutical applications etc.

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