Biological Sciences Section

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Studies on the Proteolytic Activity of Papaya Juice

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The activity of proteases in crude papaya juice *Carrica papaya* was studied by using casein as a substrate. The activity of enzyme in papaya juice was optimum at pH 8.0 and temperature 80-85°C. The crude juice was heat stable and exhibited about 8-10 fold increase in activity at 85°C. Cysteine hydrochloride and sodium cyanide showed a marked activating effect at optimum temperature.

Key words: Papaya juice, Plant proteases, Proteolytic activity, Activating agents.

Introduction

Proteolytic enzymes are abundant in vegetable kingdom and have been extracted from many plant sources (Ball and Hans 1939). For example bromelin from pineapple (Green 1975) ficin from figs (Walti 1938), papain from papaya latex (West *et al* 1966) and carboxy peptidase from wheat (Umetsu *et al* 1988), pumpkin (Green 1975), yeast (Berg 1955) and fruits of *Withania coagulans* (Amir *et al* 1970) are also good sources of proteases.

Papaya commonly grows in tropical regions. In Pakistan it is grown in Sindh and Baluchistan. Unripe papaya fruit has been in use as meat tenderizer since long time (Arnold 1975). It is some times called vegetable pepsin. This fruit is also used as a medicine in the treatment of digestive disturbances and sloughing wounds (Berg 1955).

In general, plant proteases are difficult to purify as compared to those of animal origin. A high degree of purity of papain has been reported in papaya latex and its isolation and purification is relatively simple. The papaya latex may be compared with gastric or pancreatic juice.

The proteolytic enzyme papain, in food grade quality, available for industrial use is very expensive. The specific interest has been taken in extracting papaya juice as a source of proteolytic enzyme from unripe papaya because it is abundantly available in Karachi during the months of October to March. Attempts have also been made to obtain purified enzyme from papaya juice (Kling *et al* 1982). The use of solvents for purification makes it expensive, whereas, papaya juice if used as such will prove to be a cheaper source of enzyme. It can be easily adjusted in our country's environment because it is heat stable and shows optimum activity at high temperature. The present ivestigation reports the proteolytic activity and kinetics of crude papaya juice and the factors affecting the reaction rate of enzyme (papaya juice). Enzyme actions such as enzyme concentration, temperature, substrate concentration, stability against heat treatment and effect of activating agents have been determined.

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Materials and Methods

Extraction of Papaya Juice. Fresh unripe papaya were purchased from local market, washed, cut into small pieces and juice was extracted in electric juicer MJ-280 N (National Matsushita). The extracted juice was filtered through a cotton plug to remove fine pulp. The clear juice was kept in deep-freezer before the experiment. The yield of juice obtained was about 50% (v/w) of the unripe papaya i.e. 1 kg raw papaya yielded about 400-500 ml of juice (Table1).

Measurement of Proteolytic Activity. For the the mea-

Table 1
Proteolytic activity and yield of papaya juice from 1 kg raw papaya

Samples (c)	Yield juice (ml)	Proteolytic activity (b) (u mole/ml)		%Dry matter of juice (a)	Specific gravity of juice (g/ml)
		40°C	85°C		
Oct. 94	430	1.88	17.2	4.16	1.015
Nov. 94	480	3.60	22.54	5.24	1.018
Dec. 94	470	2.50	13.86	4.30	1.013
Jan. 95	520	2.03	16.40	4.80	1.011
Feb. 95	530	3.75	20.88	5.20	1.014
March 95	490	2.95	25.45	5.70	1.016

a = Papaya juice sample averaged 4.9% (v/w) dry matter at 80°C, constant weight and pH within 5.8-59. b = units as defined in materials and methods. c = average of 50 determinations with S.D. ±.

surement of proteolytic activity (Cheftal *et al* 1971) the substrate used was casein (0.6%), Art 2242 Merck, (according to Hammarsten) in 0.2 M phosphate buffer pH 6.0. 1:20 dilution of papaya juice was made (1ml of papaya juice was activated by adding 10 drops of 2M NaCN ((Anson 1938) or 0.2 ml of 0.02 M cysteine hydrochloride (Conrat 1957) and the volume was made to 20 ml). One ml of this papaya juice dilution was added to 5 ml of substrate solution. The mixture was incubated for 10 min. at 40°C, 5 ml of 10% trichloroacetic acid (TCA) was added to prevent further hydrolysis and it was centrifuged 1 ml of 1.3 M Na_2CO_3 and 1 ml of 1:3 diluted Folin ciocalteau reagent (David 1955) were added to 2 ml of supernatant The mixture was allowed to stand for 30 min. was at 30°C and absorbance was measured at 660 nm. The blank contained the enzyme substrate mixture to which tricholoroacetic acid (TCA) was added before the start of incubation. The blank solutions were also incubated as were the test solutions.

Units of proteolytic activity. One protease unit was defined as the amount of enzyme which will hydrolyse casein in order to produce a colour (Folin Ciocalteacu) equivalent to one micro mole of tyrosine ml^{-1} $minute^{-1}$ under the assay condition.

Results and Discussion

Effect of Enzyme Concentration on Activity. Figure 1 shows the effect of enzyme concentration on proteolytic activity at pH 6 with casein as substrate. The concentration of casein was 0.03g $5ml^{-1}$. The papaya juice was prepared in different dilutions with 0.01-0.12 ml papaya juice per ml of 0.2 M phoshphate buffer and incubated at 40°C for 10 min. The activity was linear with respect to the volume of enzyme used up to the value of 0.06 ml dilution.

Effect of Temperature on Activity. The effect of temperature on the proteolytic activity is shown in (Fig 2). The concentration of casein was 0.03g $5ml^{-1}$ and dilution papaya juice was 1ml in phosphate buffer (pH 6). It was incubated for 10 min. at temp. 40-100°C. Maximum proteolytic activity was obtained at 85°C. It has been reoprted that plant proteases are resistant and often maintain their activity at elevated temperatures upto 80°C. At temp. above 80°C the activity is rapidly lost (Arnold 1975). Papaya juice showed optimum activity at 85°C, about 8 fold as much as compared to 40°C. The temperature coefficient Q_{10} (West *et al* 1966) is the ratio of reaction rate at T°C + 10°C and the T°C; Q_{10} shows the increase in reaction rate for 10°C increase in temperature. Q_{10} values showed significant correlation between the temperature rise and activity of enzyme. The Q_{10} values between three consecutive temperature were found to be 2.10±.This shows normal enzyme behaviour towards the effect of temperature.

Effect of pH on Activity. The effect of pH on proteolytic activity was measured by using 0.2 M citrate, 0.2 M phosphate and 0.2 M borate buffer at pH values ranging from 5-10. The concentration of casein was 0.03g/5ml to which was added diluted papaya juice (1ml) and incubated at 40°C for 10 min. The papaya juice showed maximum activity at pH 8.0 and it was less active at pH 9.0 (Fig 3). The result reveals that at pH values above 6.0, the dissociation of amino group becomes significant and an increase in activity is obtained from neutral to slightly alkaline pH.

Effect of Substrate Concentration on the Rate of Enzyme Action. The enzyme concentration was kept constant and the relation between the rate of enzyme activity and concentration of the substrate was examined. The reaction mixture contained 5 ml of casein substrate solution of various concentrations, prepared from a stock casein solu-

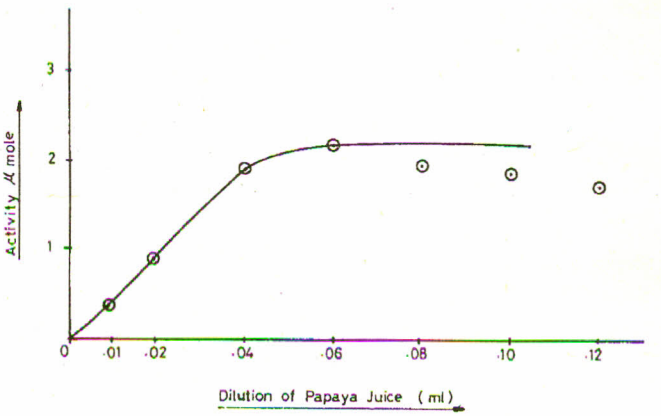

Fig 1. Effect of enzyme papaya juice concentration on activity at pH 6.0.

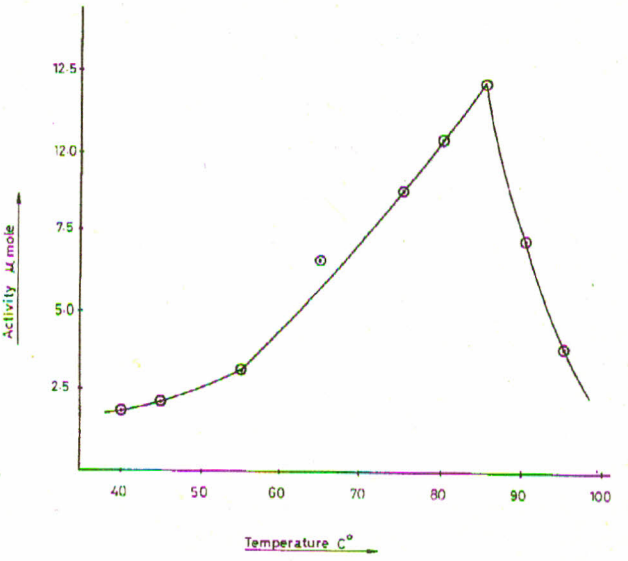

Fig 2. Effect of temperature on papaya juice activity at pH 6.0.

tion of 0.5×10^{6} M strength in 0.2 M phosphate buffer. The amount of tyrosine released was determined. Michaelis Menten Constant (Km) for tyrosine released was calculated by method of Lineweaver and Burk (Line weaver and Burk 1934). Figure 4 shows the relatonship between substrate concentration and the enzyme activiy. The Km of crude papaya juice for the substrate was found to be $1.06 \times 10\text{-}^{5}$M.

Effect of Time on Activity. The casein substrate (0.03g per 5ml) was mixed with diluted papaya juice (1ml) in 0.2 M phosphate buffer (pH 6.0). The solution was incubated at 40°C for 10, 20, 30, 40 50 and 60 min. then mixed with 5 ml of 10 % TCA. The decrease in activity was observed with the increase of the period of incubation (Fig. 5).

Effect of Activating Agents. Activation of papaya juice with cysteine hydrochloride (0.02M) and sodium cyanide (0.005M) (Anson 1938) showed a marked effect on the proteolytic activity. It has been reported that some enzymes contain sulfhydryl group which are essential for their activity and need some reducing agents like mercaptoethanols, thioglycolate, cysteine or cyanide at pH 5-7 (Conrat 1957). Cysteine hydrochloride and NaCN at 40° C doubled the activity, whereas, at optimum temperature of 85°C, about 8 fold increase in activity was observed.

Stability Against Heat Treatment. Freshly extracted papaya juice was heated at temperatures of 40,60, 70, 80, 85, 95 and 100°C for 5 min, immediately cooled in ice water and

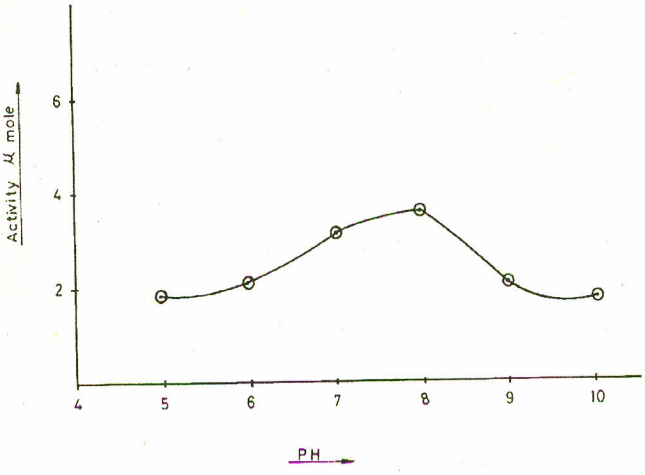

Fig 3. Effect of pH on papaya juice activity.

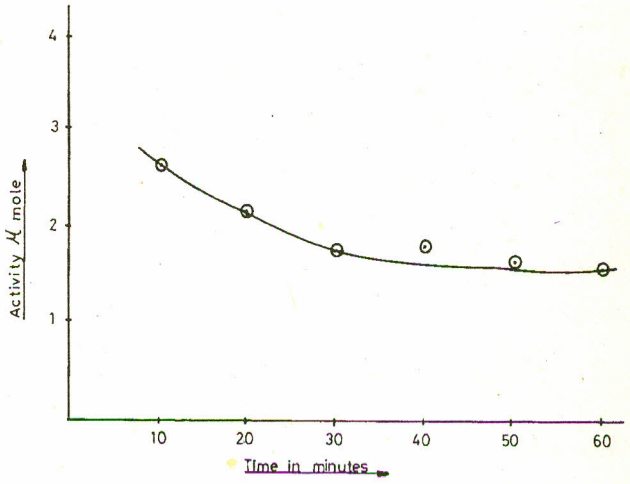

Fig 5. Effect of time on papaya juice activity at pH 6.0.

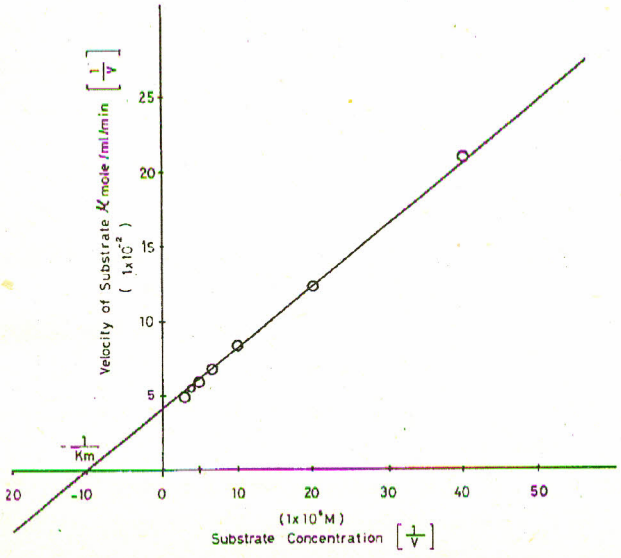

Fig 4. Effect of substrate concentration on papaya juice activity.

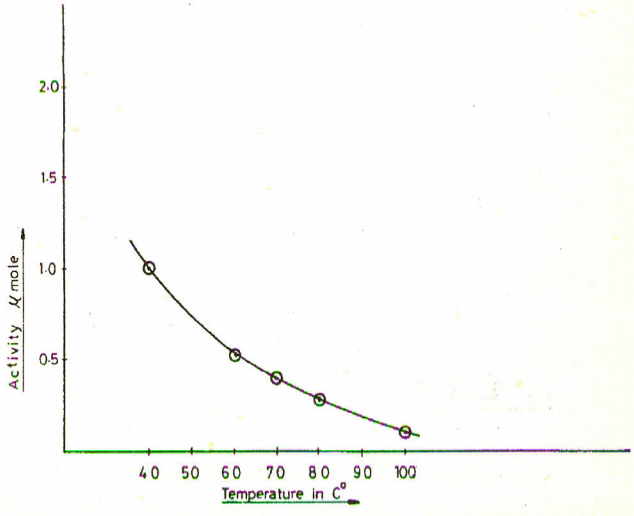

Fig 6. Stability of papaya juice against heat treatment (not activated).

the residual activity was determined at 40°C. The results are shown in Fig 6. The papaya juice was de-activated by heating especially at elevated temperatures and became completely inactive at 100°C.

In a similar experiment after heating and cooling papaya juice, 1 ml solutions were activated by 0.02M cysteine hydrochloride (0.5ml) and the residual activity was determined at 85°C. The results are shown in Fig 7. The activity is affected by heat even at low temperature but it is still higher than inactivated juice incubated at 40°C. The proteolytic activity increased about 6 folds after heating at 40-80°C. These results reveal that the activity of fresh papaya juice when kept at elevated temperatures even for 5 min. is gradually lost and the juice is completely inactivated at 100°C. The activation of the same juice shows the same pattern of denaturation but the residual activity increases about 6 folds.

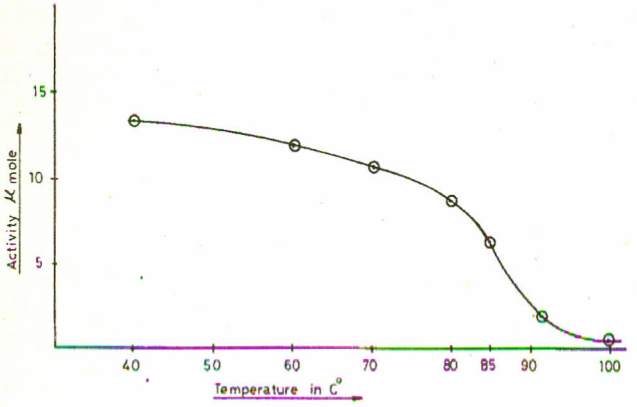

Fig 7. Stability of papaya juice against heat treatment activated with 0.02 m Cystein HCl.

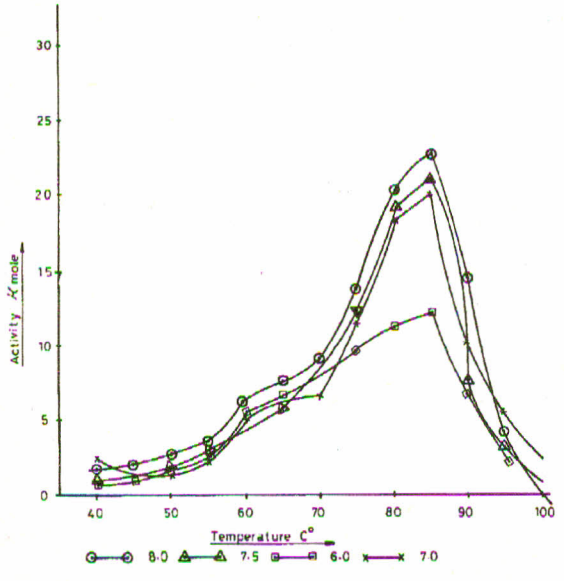

Fig 8. Optimum temperature and pH of papaya juice.

Conclusion

The optimum conditions for the hydrolysis of protein with papaya juice (Fig 8) are as follows:

Enzyme/substrate	:	1:20
pH	:	7-8
Temperature	:	80-85°C

Cysteine hydrochloride (0.02M) was used for activation of papaya juice before initiation of hydrolysis. It is a very good reducing agent for disulphide bonds of the protein (Putman 1953). It was shown that the papaya juice contained s-s linkages, which reduced to -SH groups and may be responsible for activation. Activity of papaya juice directly depends on these groups which are located at or near the enzyme site. These groups increase the activity when meet some reducing agents like mercaptan, thioglycolate, cyanide ect.

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