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*RESEARCH ARTICLE*

# Enzymological characterization of pineapple extract for potential application in oak tasar (*Antheraea proylei* J.) silk cocoon cooking and reeling

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**Keywords:** cysteine proteinase, pineapple fruit extract, proteinase activity, tasar silk industry.

**Abbreviations:**
TCA: trichloroacetic acid;
NBFL: non-breakable filament length

**Proteinases have the potential to effect partial solubilization of the proteinaceous gum sericin involved in binding the silk strands together in cocoon, an essential step in the silk cocoon cooking and reeling. Therefore, pineapple extract rich in cysteine proteinases was enzymologically characterized for its potential application in oak tasar (*Antheraea proylei* J.) silk cocoon cooking and reeling. Optimum sodium carbonate concentration (9.8 mM) and optimum temperature (60ºC) for the proteinase activity were determined. Though relatively thermostable, an enhanced activity loss was observed when the extract was incubated in the temperature range 70-90ºC with sodium carbonate. Bulk of the activity (80-83%) remained after 1 hr of time-dependent inactivation at 60ºC. The tasar cocoon extract neither caused inhibition of the activity nor enhanced its time-dependent loss by incubation at 60ºC. However, it caused an enhanced time-dependent loss of the activity by incubation at 60ºC with sodium carbonate. Considering these enzymological characteristics, experimental cocoon-cooking media were constituted by taking the pineapple extract with or without sodium carbonate at room temperature or 60ºC. The results of the cocoon cooking and subsequent single silk filament reeling indicated for an applicability of pineapple extract as an effective agent for the oak tasar cocoon cooking and reeling.**

The fruit of pineapple, *Ananas comosus* (L.) Merr. is a rich source of a mixture of cysteine proteinases, the most abundant among them being the fruit bromelain (EC 3.4.22.33) which hydrolytically cleaves the internal peptide bonds in proteins with relatively broad specificity (Rowan and Buttle, 1994). The pineapple proteinases find uses in various industrial and medical applications including brewing, meat tenderization, prevention of diarrhea, digestive aids and treatment of edema (Takagi et al. 1992; Tanabe et al. 1996; Chandler and Mynott, 1998; Kelly,

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1996; Maurer, 2001). The use of enzymes in the silk industry is relatively unexplored, has generated a lot of interest, and much research is being carried out internationally (Gulrajani et al. 2000). Against this background, pineapple fruit pulp extract (simply pineapple extract) having proteinase activity may also find application in tasar silk industry in general and tasar silk cocoon cooking and reeling in particular. The oak tasar (*Antheraea proylei* J.) silkworm is an important source of tasar silk (Singh and Singh, 1998). The larvae of the silkworm, reared on leaves of oak tree *Quercus* species (Family-Fagaceae), produce the cocoons from which the tasar silk is reeled (Singh and Singh, 1998). Unlike the mulberry (*Bombyx mori* L.) silk cocoons, the oak tasar silk cocoons cannot be satisfactorily softened by boiling in plain water (Jolly et al. 1979). The cocoons containing relatively higher amounts of protein-tannin complexes in the form of proanthocyanidins (Pandey and Goel, 1990; Pandey, 1997), have to be softened by more drastic boiling off techniques (Pandey and Goel, 1990). Generally the cocoons are cooked in presence of strong alkali agent or other harsh chemicals (Tikoo and Goel, 1987; Das, 1993; Iizuka et al. 1993; Moon et al. 1996; Chattopadhyay et al. 1997). Since the chemical methods reduce the quality of the tasar silk thread in many ways (Tikoo and Goel, 1987), an alternative method for the oak tasar cocoon cooking based on the proteolytically active pineapple extract may be developed for better results. The pineapple extract may be used in cooking of the silk cocoon to soften it by decomposing or partially solubilizing the proteinaceous silk gum sericin which is involved in binding the fibroin silk strands together in the cocoon shell. In the present investigation, a study was undertaken to enzymologically characterize the pineapple extract having proteinase activity with the aim of developing an effective oak tasar (*Antheraea proylei* J.) silk cocoon cooking method based on pineapple extract.

## MATERIALS AND METHODS

### Pineapple pulp

Fresh and ripe (mature and yellow) fruit of pineapple *Ananas comosus* (L.) Merr. *cv.* Queen was purchased from the markets in and around Imphal, Manipur, India. The pineapple fruit pulp was prepared from the fruit by first detaching the crown and stem parts, and then slicing off the skin part.

### Silk cocoon

The cocoons produced by the oak tasar silkworm *Antheraea proylei* J. fed on *Quercus serrata* (Thunb.) leaves, hot air stifled for 6-7 hrs at 70ºC, and then stored for 2-3 months were used in the present investigation. Three specimen cocoons are shown in Figure 1. The cocoons were obtained from Regional Tasar Research Station, Imphal, India.

### Preparation of pineapple extract

The pulp (15 g) of ripe (mature and yellow) pineapple fruit was homogenized with distilled water (100 mL). The resulting homogenate was strained through a coarse cotton cloth and then centrifuged (13,000 xg at 4ºC for 10 min) to collect the supernatant as the pineapple extract having proteinase activity.

### Proteinase assay

The proteinase activity of the pineapple extract was assayed by a modification of the azocasein method of Rowan and Buttle, 1994 in which the time-dependent release of azo-dye-coupled-TCA-soluble-peptide fragments from the proteinase substrate azocasein was monitored. The assay mixture was constituted by mixing 1.0 mL of the pineapple extract with 0.2 mL of 0.3% (w/v) azocasein at 30ºC. The assay mixture was found to have a fixed pH value ranging from 3.8 to 4.4 which lies within a buffering range of the pineapple extract. The reaction was started with the addition of the substrate. After 30 min interval, the reaction was arrested by adding 0.3 mL of 44.4% (w/v) TCA. The protein precipitation was allowed to complete by cooling the resulting reaction mixture in ice for 5 min and then centrifuged to collect the supernatant. 1.0 mL of the resulting acidic supernatant was first mixed with 0.7 mL of 1.0 M NaOH, and then absorbance at 420 nm was read. A corresponding blank was run by adding TCA to the pineapple extract prior to the mixing with the azocasein solution.

### Determination of effect of sodium carbonate

The proteinase activity of the pineapple extract was determined as a function of sodium carbonate concentration. The proteinase assay mixture was constituted by mixing 1.0 mL of the pineapple extract containing different amounts of sodium carbonate with 0.2 mL of 0.3% (w/v) azocasein at 30ºC. After 30 min interval, the reaction was arrested by adding 0.3 mL of 44.4% (w/v) TCA. The rest of the procedure was same as in Proteinase assay. Corresponding blank at each concentration of sodium carbonate was run by adding TCA to the pineapple extract prior to mixing with azocasein.

### Determination of optimum temperature

The proteinase activity of the pineapple extract was determined as a function of temperature. The assay mixture, constituted by mixing 1.0 mL of the pineapple extract with 0.2 mL of 0.3% (w/v) azocasein, was incubated at different designated temperatures using a thermostat (sensitivity ± 0.01ºC). After 30 min incubation, the reaction was arrested by addition of 0.3 mL of 44.4% (w/v) TCA. The rest of the procedure was same as in Proteinase assay. Corresponding blank at each temperature was run by adding TCA to the pineapple extract prior to mixing with azocasein. Similar experiment was repeated with reaction mixture containing 9.8 mM sodium carbonate.

### Determination of stability characteristics

**Thermal stability.** 1.0 mL of the extract taken in a microfuge tube was incubated at different designated temperatures using a thermostat (sensitivity ± 0.01ºC). After 30 min, the extract was cooled in ice for 5 min, brought to and maintained at 30°C, and then the proteinase activity was assayed as usual. Corresponding blank at each temperature was run by adding TCA prior to addition of azocasein to the pineapple extract. Similar experiment was repeated by incubating the pineapple extract containing 9.8 mM sodium carbonate at different temperatures.

**Time course of inactivation.** The time course of loss of the proteinase activity in the pineapple extract was determined by incubating 20 mL of it at 60ºC in a thermostat (sensitivity ± 0.01ºC). At different time intervals, 1.0 mL aliquot was withdrawn, brought to and maintained at 30ºC, and then the proteinase activity was determined as usual. Corresponding blank at each time interval was run by adding TCA prior to addition of azocasein to the pineapple extract. Similar experiment was repeated by taking the pineapple extract containing 9.8 mM sodium carbonate.

### Determination of effect of cocoon extract

**Effect on proteinase activity.** Ten pieces of the hot air stifled good oak tasar cocoon were boiled with 1 L of distilled water for 30 min and the brown extract obtained was used in place of distilled water for preparation of pineapple extract. 7.5 g of pineapple fruit pulp was homogenized with 50 mL of the cocoon extract. The resulting homogenate was strained through a coarse cotton cloth and then centrifuged (13,000 xg for 10 min at 4ºC) to collect the supernatant as the pineapple extract prepared in cocoon extract. Proteinase activity in the pineapple extract was determined as usual. A corresponding blank was run by adding TCA to the pineapple extract prior to the mixing with the azocasein solution. The activity was also determined by preparing the assay mixture containing 9.8 mM sodium carbonate. Both the proteinase assays in absence and presence of sodium carbonate were repeated by incubating the assay mixture at 60ºC.

**Effect on time course of inactivation.** For determining the time course of loss of the proteinase activity in the pineapple extract prepared in cocoon extract, 20 mL of the extract was incubated at 60ºC. At different time intervals, 1.0 mL aliquot was withdrawn and its proteinase activity was determined as usual. Corresponding blank at each time interval was run by adding TCA prior to addition of azocasein to the pineapple extract.

### Testing for applicability of pineapple extract in oak tasar cocoon cooking and reeling

Ninety pieces of hot air stifled good oak tasar (*Antheraea proylei* J.) silk cocoon were initially boiled for 30 min in distilled water taking care that the cocoons always remained completely dipped in the boiling water. 15 of the boiled cocoons were soaked in distilled water at room temperature (26-31ºC) for 20 hr, and another 15 were soaked in distilled water at 60ºC for 4 hrs. In a parallel set of experiments, 15 of the boiled cocoons were soaked in the pineapple extract at room temperature (26-31ºC) for 20 hr, and another 15 were soaked in the extract at 60ºC for 4 hrs. Yet, in another parallel set of experiments, 15 of the boiled cocoons were soaked in the pineapple extract containing 9.8 mM sodium carbonate at room temperature (26-31ºC) for 6 hrs, and another 15 were soaked in the same soaking medium at 60ºC for 0.5 hrs. At the completion of each of the soaking step, the cocoon samples were taken out, washed in tap water, semi-dried, deflossed, and then single silk filament reeling was performed on an epprouvette machine in the Reeling Section, Regional Tasar Research Station, Imphal, India.

## RESULTS AND DISCUSSION

The proteinase assay mixture, constituted by simply mixing the pineapple extract from ripe (mature and yellow) fruit with azocasein solution in the absence of any externally added buffer, was found to have a fixed pH value ranging from 3.8 to 4.4, which is within a buffering range of the pineapple extract inferred from its titration curve shown in Figure 2. Only the pineapple extract from ripe (mature and yellow) fruit was used in the present investigation as its proteinase activity was found to be about 30% higher than the corresponding extract from unripe (mature but green) fruit (experimental results not shown). Addition of sodium carbonate in the assay mixture was found to have an enhancing effect on the proteinase activity. The results are shown in Figure 3. The optimum sodium carbonate concentration for expression of maximum activity was found to be 9.8 mM (0.125%). The result suggested that the pineapple extract containing 9.8 mM sodium carbonate might be better employed than the extract alone where higher proteinase activity is required. The results of the experimental study on the effect of temperature on the proteinase activity of the pineapple extract are shown in Figure 4. The optimum temperature was found to be 60ºC either in the absence or presence of 9.8 mM sodium carbonate. A higher activity was however observed in the presence than in the absence of sodium carbonate in whole of the temperature range 30-90ºC, the increase in the activity being more in the lower temperature range than in the higher temperature range. The results of the experimental study on the thermal stability behaviour of the proteinase activity in pineapple extract are given in Figure 5. Only up to 13% of the activity was lost when the extract was incubated for 30 min at designated temperatures up to 60ºC in the absence of sodium carbonate. The result was found to be consistent with that reported earlier (Greenberg, 1955). At higher temperatures, the activity was lost faster. However, 20% residual activity remained even after 90ºC incubation for 30 min. The overall thermal stability of the proteinase activity in the presence of 9.8 mM sodium carbonate was more or less the same when compared with

that of the pineapple extract alone in the incubation temperature range 30-60ºC. However, the thermal stability was found to be appreciably lower in the presence of sodium carbonate in the higher incubation temperature range 70-90ºC. After 90ºC incubation for 30 min, only 6% of the residual activity remained. Although nothing definite could be said to explain the observed enhanced thermal destabilization in presence of sodium carbonate specially when dealing with a crude proteinase preparation, an effect of sodium carbonate on the role of some chaperoning proteins, that could determine the secondary structure of cysteine proteinases of the pineapple extract, might be speculated. The time courses of loss of the proteinase activity in pineapple extract maintained at 60ºC in the absence or presence of 9.8 mM sodium carbonate are shown in Figure 6. The proteinase activity was lost relatively slowly retaining 80-83% activity after incubation for 1 hr at 60ºC either in the absence or presence of 9.8 mM sodium carbonate. The overall stability of the proteinase activity at 60ºC was only slightly lower in presence than in absence of the sodium carbonate concentration.

Boiling of the oak tasar cocoon in water yielded a brown coloured extract. The effects of the cocoon extract on the proteinase activity in the pineapple extract and on its time course of thermal inactivation were studied. The results are given in Table 1 where a comparison is presented between the pineapple extract prepared in distilled water and that prepared in cocoon extract with respect to their proteinase activity and thermal inactivation behaviour under various experimental conditions. The cocoon extract was found to have little effect on the proteinase activity of the pineapple extract either at room temperature (26-31ºC) or at 60ºC in the absence or presence of 9.8 mM sodium carbonate indicating thereby that the brown materials released from the oak tasar cocoon during its boiling do not inhibit the proteinase activity. With respect to the time course of thermal inactivation, it was found that the cocoon extract does not appreciably alter the rate of loss of the proteinase activity at 60ºC in the absence of sodium carbonate. On the other hand, the proteinase activity was lost faster in the presence of 9.8 mM sodium carbonate under the same experimental conditions. The materials in the brown coloured cocoon extract in combination with sodium carbonate could be responsible for a destabilization of the proteinases of the pineapple extract. More than 80% of the activity, however, remained after half-an hour incubation of the pineapple extract prepared in cocoon extract in presence of 9.8 mM sodium carbonate at 60ºC.

By considering the above enzymological characteristics of the pineapple extract with respect to its proteinase activity, different oak tasar silk cocoon cooking media were constituted by taking the pineapple extract with or without 9.8 mM sodium carbonate at room temperature (26-31ºC) or 60ºC. The results of an experimental study on the applicability of the constituted cooking media in oak tasar silk cocoon cooking and subsequent single silk filament reeling on an epprouvette machine are given in Table 2. No single filament reeling could be performed due to lack of minimal softening of the cocoons soaked in distilled water for 20 hrs at room temperature (26-31ºC) or for 4 hrs at 60ºC following the initial 30 min boiling. On the other hand, the cocoons soaked in the pineapple extract for 20 hrs at room temperature (26-31ºC) or for 4 hrs at 60ºC following the initial 30 min boiling were softened at least minimally and they could be subjected to single silk filament reeling. The two different cocoon cooking procedures involving soaking in pineapple extract gave more or less the same reeling performances monitored by the reeling parameters - number of ends feeding /cocoon, filament length, recovery %, NBFL and reelability %. The overall reeling performance was further improved by incorporating 9.8 mM sodium carbonate into the pineapple extract cocoon-cooking medium. Besides the improvement in the reeling performances, the overall cocoon cooking time was markedly reduced by incorporating 9.8 mM sodium carbonate into the pineapple extract cooking media (basic overall cooking time of 6.5 hrs as against 20.5 hrs in absence of sodium carbonate at room temperature; basic overall cooking time of 1 hr as against 4.5 hrs in absence of sodium carbonate at 60ºC). These experimental results were suggestive for an applicability of pineapple extract with or without 9.8 mM sodium carbonate as an effective agent for oak tasar (*Antheraea proylei* J.) silk cocoon cooking and reeling. Further detailed experimental study is underway for development of a simple but effective oak tasar silk cocoon cooking method using pineapple extract which is not only readily accessible to common tasar silk reelers and weavers but also advantageous in many aspects of post cocoon technology in tasar silk industry.

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## REFERENCES

CHANDLER, D.S. and MYNOTT, T.L. Bromelain protects piglets from diarrhea caused by oral challenge with K88 positive enterotoxigenic *Escherichia coli*. *Gut*, 1998, vol. 43, no. 2, p. 196-202.

CHATTOPADHYAY, R.; DAS, S.; GULRAJANI, M.L. and SEN, K. A study on the progressive change in characteristics of the bave (filament) along its length in mulberry and tasar cocoons. *Sericologia*, 1997, vol. 37, no. 2, p. 263-270.

DAS, S. Chemical processing of tasar silk. In: GULRAJANI, M.L. ed. *Chemical processing of silk*. Delhi, Department of Textile Technology, Indian Institute of Technology, 1993, p. 46-62.

GREENBERG, D.M. Plant proteolytic enzymes. In: COLOWICK, S.P. and KAPLAN, N.O., eds. *Methods in Enzymology*. New York, USA, Academic Press Inc., 1955, vol. 1, p. 54-64.

GULRAJANI, M.L.; GROVER, A.; SURI, M. and AGRAWAL, R. Degumming of silk with lipase and protease. *Indian Journal of Fibre and Textile Research*, 2000, vol. 25, no. 1, p. 69-74.

IIZUKA, E.; KAWANO, R.; KITANI, Y.; OKACHI, Y.; SHIMIZU, M. and FAKUDA, A. Studies on the physical properties of Indian non-mulberry silks I, *Antheraea proylei*. J. *Indian Journal of Sericulture,* 1993, vol. 32, no. 1, p. 27-36.

JOLLY, M.S.; SEN, S.K.; SONWALKAR, T.N. and PRASAD G.K. Non mulberry silks. *Agricultural Services Bulletin*, Rome, FAO of the United Nations, 1979.

KELLY, G.S. Bromelain: a literature review and discussion of its therapeutic applications. *Alernative Medicine Review*, 1996, vol. 1, no. 4, p. 243-257.

LEE, Y.W. Silk reeling and testing manual. Agricultural Services Bulletin no. 136, Rome, FAO of the United Nations,1999.

MAURER, HR. Bromelain: biochemistry, pharmacology and medical use. *Cellular and Molecular Life Sciences*, 2001, vol. 58, no. 9, p.1234-1245.

MOON, M.A.; SENGUPTA, D. and SINHA, S.S. Hydrogen peroxide: a safe and effective cocoon softening agent. *Indian Silk*, 1996, vol. 34, no. 12, p. 21-22.

PANDEY, R.K. Adaptive significance of gut pH in the oak tasar silkworm, *Antheraea proylei* J. *Sericologia*, 1997, vol. 37, no. 1, p. 157-160.

PANDEY, R.K. and GOEL, R.K. Pro-anthocyanidins in the cocoon shell of 3 *Antheraea* species. *Sericologia*, 1990, vol. 30, no. 4, p. 527-529.

ROWAN, A.D. and BUTTLE, D.J. Pineapple cysteine endopeptidases. In: BARRETT, A.J., ed. *Methods in Enzymology*. New York, USA, Academic Press Inc., 1994, vol. 244, p. 555-568.

SINGH, K.C. and SINGH, N.I. Biology and ecology of temperate tasar silkmoths, *Antheraea proylei* Jolly and *Antheraea pernyi* Guerin-Meneville (Saturniidae): a review. *Indian Journal of Sericulture*, 1998, vol. 37, no. 2, p. 89-100.

TAKAGI, H.; KONDOU, M.; HISATSUKA, T.; NAKAMORI, S.; TSAI, Y.C. and YAMASAKI, M. Effects of an alkaline elastase from an alkalophilic bacillus strain on the tenderization of beef meat. *Journal of Agricultural and Food Chemistry*, 1992, vol. 40, no. 12, p. 2364-2368.

TANABE, S.; ARAI, S. and WATANABE, M. Modification of wheat flour with bromelain and baking hypoallergenic bread with added ingredients. *Bioscience, Biotechnology and Biochemistry*, 1996, vol. 60, no. 8, p. 1269-1272.

TIKOO, B.L. and GOEL, R.K. Oak tasar cocoon: development of a simple cooking method. *Indian Silk*, 1987, vol. 25, no. 9, p. 55-56.

# APPENDIX

## Tables

**Table 1: Comparison between the pineapple extract prepared in distilled water and that prepared in cocoon extract with respect to proteinase activity and thermal inactivation behaviour.**

| Pineapple extract | | Proteinase activity (ΔAbs at 420 nm / 30 min)* | | % Residual activity of thermal inactivation at 60ºC | |
|---|---|---|---|---|---|
| | | At room temperature (26-31ºC) | At 60ºC | After 30 min incubation | After 60 min incubation |
| Pineapple extract prepared in distilled water | Without sodium carbonate | 0.146 | 0.357 | 87.2 | 82.6 |
| | With 9.8 mM sodium carbonate | 0.350 | 0.399 | 95.2 | 79.9 |
| Pineapple extract prepared in cocoon extract | Without sodium carbonate | 0.167 | 0.312 | 93.8 | 83.1 |
| | With 9.8 mM sodium carbonate | 0.327 | 0.408 | 81.4 | 51.4 |

*The proteinase activity in pineapple extract was assayed by a modification of the azocasein method of Rowan and Buttle, 1994 described in Proteinase assay given in the text.

**Table 2: Results of experimental study on the applicability of pineapple extract in oak tasar (*Antheraea proylei* J.) cocoon cooking and reeling*.**

| **Initial cocoon treatment** | 30 min boiling in distilled water | | | | | |
|---|---|---|---|---|---|---|
| **Soaking** | Soaked in distilled water at room temperature (26º-31ºC) | Soaked in distilled water at 60ºC | Soaked in pineapple extract at room temperature (26º-31ºC) | Soaked in pineapple extract at 60ºC | Soaked in pineapple extract with 9.8 mM sodium carbonate at room temperature (26º-31ºC) | Soaked in pineapple extract with 9.8 mM sodium carbonate at 60ºC |
| **Soaking time (hr)** | 20 | 4 | 20 | 4 | 6 | 0.5 |
| **Number of ends feeding / cocoon**** | No single filament reeling could be performed due to lack of minimal softening of the cocoon | No single filament reeling could be performed due to lack of minimal softening of the cocoon | 7.3 | 7.1 | 6.7 | 6.0 |
| **Filament length (m)***** | | | 679.9 | 790.0 | 703.1 | 854.7 |
| **Recovery %******** | | | 57.6 | 60.6 | 61.0 | 64.7 |
| **NBFL (m)********* | | | 93.1 | 111.4 | 104.8 | 142.7 |
| **Reelability %********** | | | 13.7 | 14.1 | 14.9 | 16.7 |

*The single silk filament reeling was performed on an epprouvette machine in the Reeling Section, Regional Tasar Research Station, Imphal, India. Each value in the table is an average of fifteen replications.
**Number of ends feeding / cocoon = Number of breaks encountered by a cocoon while reeling
*** Filament length (m) = Length of reeled silk filament per cocoon in meters
****Recovery % = (Filament weight / Cocoon shell weight) x 100
*****NBFL (m) = (Filament length in meters x Reelability %) / 100 (Lee, 1999)
******Reelability % = (Number of reeled cocoons / Number of ends feeding) x 100 (Lee, 1999)

# Figures

**Figure 1. Specimens of the oak tasar (*Antheraea proylei* J.) silk cocoon**. The cocoon is an important source of non-mulberry silk.

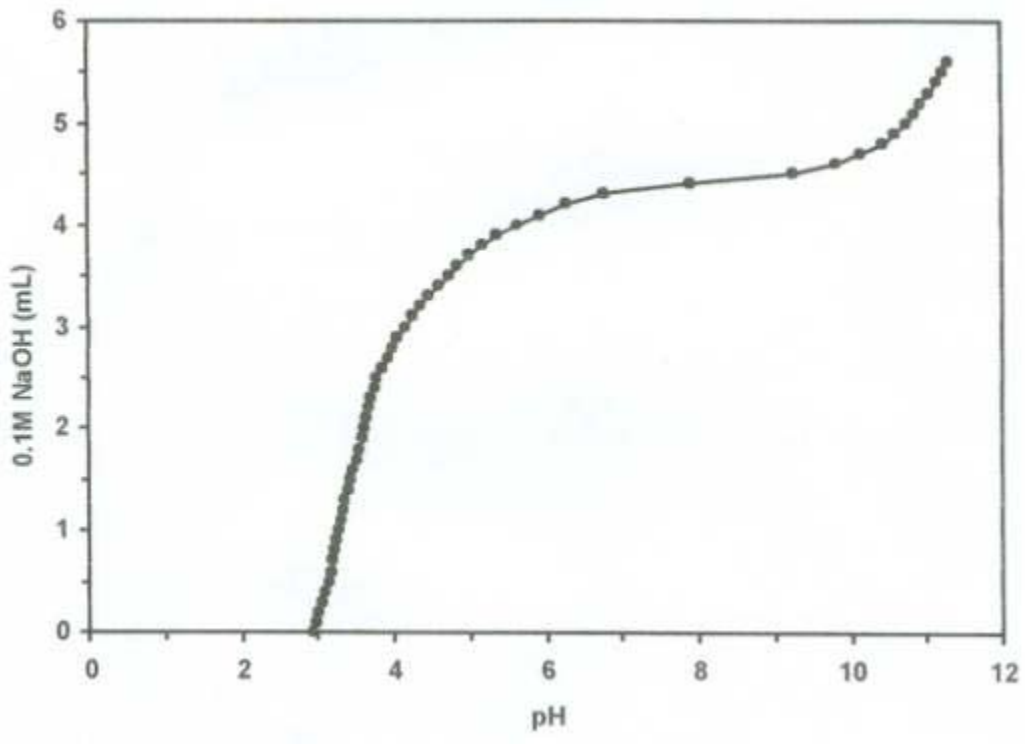


**Figure 2. Titration curve of pineapple extract.** 10 mL of the extract prepared as in 'Preparation of pineapple extract' given in the text was acidified with HCl to pH 2.95, 0.1 mL aliquots of 0.1 M NaOH were added under constant stirring, and pH was determined before and after each addition of alkali at 30ºC.

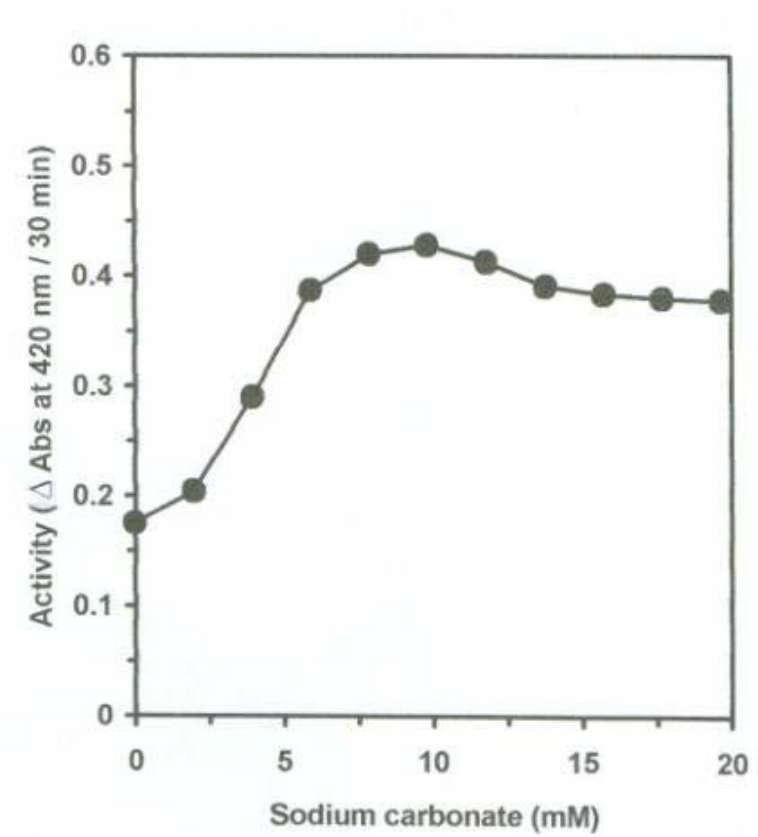


**Figure 3. Effect of sodium carbonate on the proteinase activity of pineapple extract at 30ºC.** The proteinase activity was assayed as in 'Proteinase assay' given in the text.

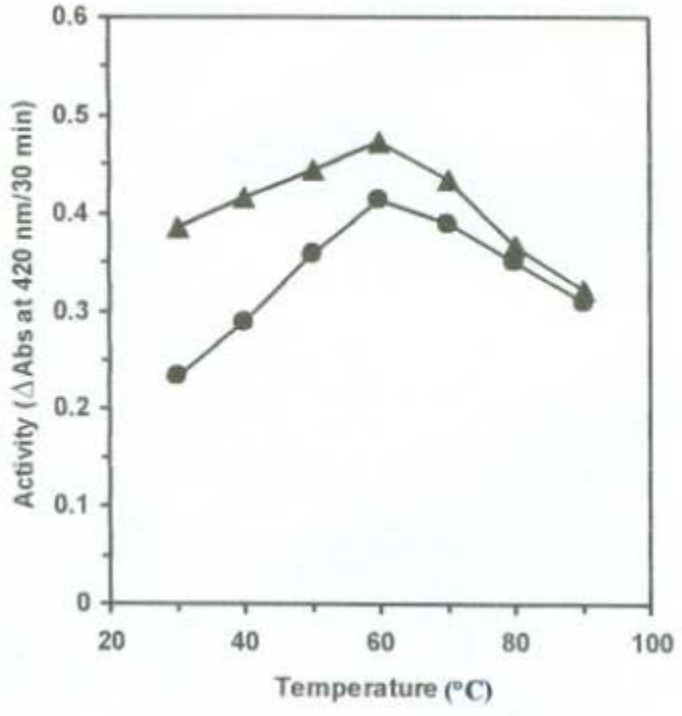


**Figure 4. Effect of temperature on the proteinase activity of pineapple extract in absence (●-●-●) and presence (▲-▲-▲) of 9.8 mM sodium carbonate.** The proteinase activity was assayed as in 'Proteinase assay' given in the text.

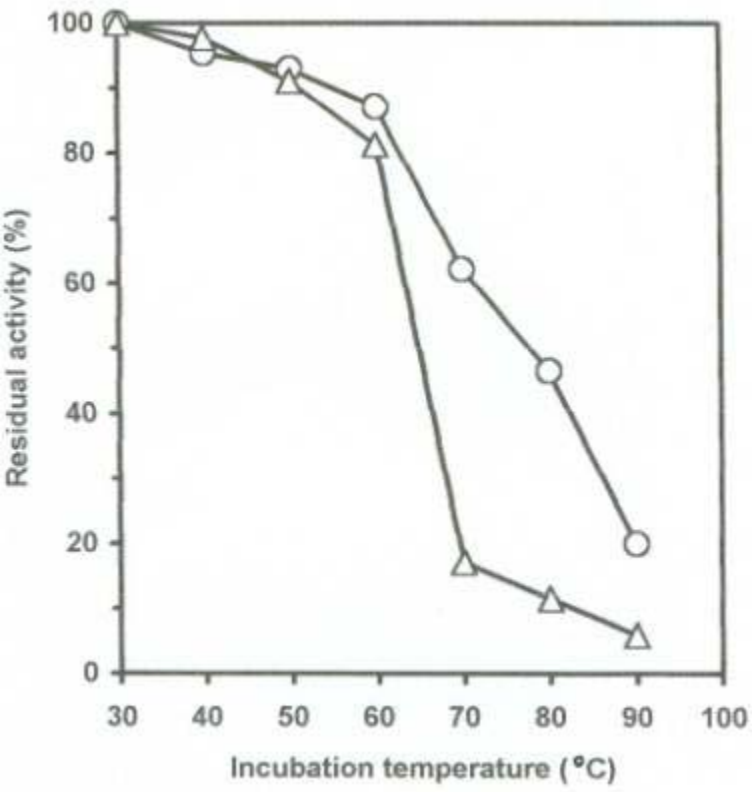


**Figure 5. Thermal stability behaviour of the proteinase activity in the pineapple extract in the absence (○-○-○) and presence (Δ-Δ-Δ) of 9.8 mM sodium carbonate.** 1.0 mL aliquots of the pineapple extract were incubated for 30 min at different designated temperatures, cooled in ice for 5 min, brought to and maintained at 30ºC, and the proteinase activity was assayed as in Proteinase assay given in the text.

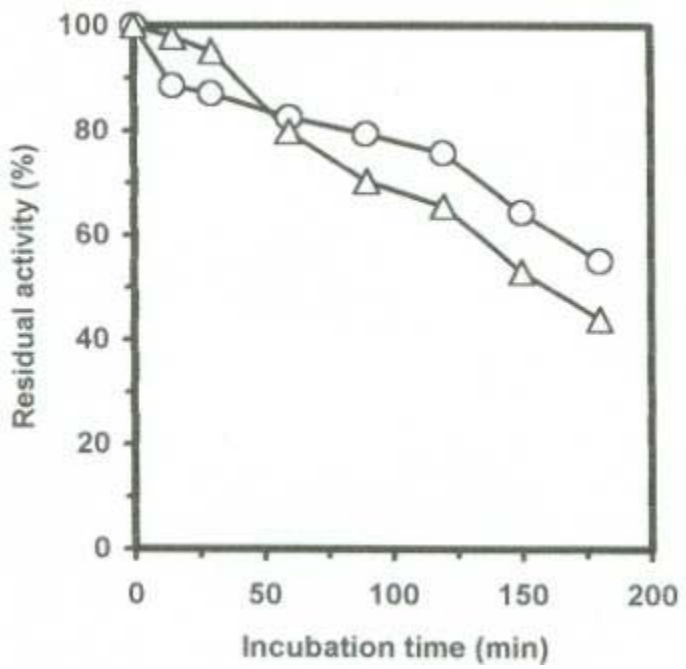


**Figure 6. Time course of inactivation of proteinase activity of pineapple extract incubated at 60ºC in the absence (○-○-○) or presence (Δ-Δ-Δ) of 9.8 mM sodium carbonate.** 1.0 mL aliquots of the pineapple extract were withdrawn at different time intervals, cooled to and maintained at 30ºC, and the proteinase activity was assayed as in Proteinase assay given in the text.