Kasetsart J. (Nat. Sci.) 44 : 943 - 948 (2010)

Effect of Temperature on the Stability of Fruit Bromelain from Smooth Cayenne Pineapple

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ABSTRACT

Fruit bromelain was extracted from the fruit of pineapples. Crude enzyme extract was subjected to heat treatment at 40-80°C for 0-60 min. The temperature stability profiles as a function of different time intervals showed higher retention of enzyme activity at low temperature. Incubation at 40°C showed no loss of fruit bromelain activity up to 60 min, whereas at 50°C almost 83% of activity remained. Incubation at 80°C for 8 min caused almost complete activity loss. Thermal inactivation of fruit bromelain in the temperature range 40-80°C was described by a first-order model. The calculated activation energy (*E*a) value for fruit bromelain was 313.18 ± 57.44 kJ/mol.

Keywords: bromelain, stability, pineapple, temperature, enzyme

INTRODUCTION

Nowadays, consumers are increasingly interested in health and nutrition, so consequently, fruits and vegetables are consumed much more than in previous years. Pineapple is a fruit that is planted all over Thailand and is generally consumed fresh. Bromelain is an enzyme that is beneficial for health and is found naturally in pineapples. It has been used for a long time as a medicinal substance by several native cultures, and has been chemically known since 1876 (Taussig and Batkin, 1988). Pharmaceutical applications of bromelain as a therapeutic compound were first used in 1957. Its actions, some of which have been recently discovered, include antitumor properties, immunity modulation, digestive assistance, enhanced wound healing, and cardiovascular and circulatory improvement (Maurer, 2001; Hale *et al*., 2005). Through their action as anti-inflammatory agents, and by increasing the permeability of the blood-brain barrier to nutrients and therapeutic agents, plant cysteine proteases, especially bromelain, have shown certain possibilities for prospective application in vivo to Alzheimer's disease patients (Lauer *et al*., 2001).

Among the studies concerning the thermal stability of bromelain, most have been performed using commercial bromelain from the stems of pineapples (Yoshioka *et al*., 1991; Arroyo-Reyna and Hernandez-Arana, 1995; Gupta *et al*., 2007). Few studies have investigated the effect of temperature on bromelain obtained from pineapple fruit.

Varieties of processed pineapple cubes and juice are widely available in the marketplace. While testing the effectiveness of these products, it was observed that all were completely devoid of proteolytic activity, as compared with the high activity found in fresh fruit extract (Bhattacharya

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Received date : 26/10/09 Accepted date : 26/04/10

and Bhattacharyya, 2007). Since the medicinal properties of pineapple have been primarily associated with the proteolytic activity of bromelain (Hale *et al*., 2005), the processed products are likely to be ineffective proteolytically. This inactivation of enzymes may arise from the harsh conditions of sterilization, precipitation and/or autodigestion. Information on the thermal stability of fruit bromelain is of great importance for pineapple processing, particularly for those who are interested in preserving bromelain activity in their products.

Commercial bromelain from pineapple stems has been found to be completely inactivated by heating for 30 min at 60°C (Yoshioka *et al*., 1991); while Gupta *et al*. (2007) found that bromelain retained 50% of its activity level after 20 min heating at 60°C. Liang *et al*. (1999) found that bromelain from pineapple fruit juice concentrate retained 50% of its initial activity after 60 min heating at 60°C. Bromelain from frozen pineapple fruit of *Bromelia balansae* Mez had no activity loss when incubated at 37°C for a period of 120 min, whereas at 45°C almost 80% of activity remained. The enzyme was almost completely inactivated by heating for 60 min at 75°C (Pardo *et al*., 2000). Valles *et al*. (2007) found that there was no bromelain enzyme activity loss when bromelain from *Bromelia antiacantha* Bertol was incubated at 37°C for 180 min, or at 55°C for 60 min, while after 30 min at 60°C, it retained 80% of its initial activity. In these studies, the researchers used different varieties of pineapples. None of the above research on bromelain from pineapple fruits reported on kinetic studies.

The *E*a value of bromelain has been reported as 174.47 kJ/mol (Yoshioka *et al*., 1991) and 181 ± 35 kJ/mol (Arroyo-Reyna and Hernandez-Arana, 1995), with all measurements based on commercial pure bromelain extracted from the stems of pineapples. The objective of the current study was to investigate the effect of temperature on the stability of fruit bromelain from Smooth Cayenne pineapple grown in Thailand. Thermal kinetic studies of fruit bromelain were also carried out.

MATERIALS AND METHODS

Raw material

Pineapples (*Ananas comosus*) of the Smooth Cayenne variety (Dole Thailand, Ltd.) were purchased from a local market. Pineapples were washed and then peeled with a borer (80 mm diameter); the cores were then removed using a smaller borer (30 mm diameter). Pineapple slices were cut into small pieces before separating the pineapple juice with a juice extractor (Hitachi Ltd., Tokyo, Japan).

Crude bromelain extract preparation

Crude bromelain extract preparation followed the method of Pardo *et al*. (2000). Crude bromelain extract was obtained by mixing pineapple juice with cold 0.1 M sodium phosphate buffer (pH 8.0) containing 5 mM EDTA and 25 mM cysteine. This was then centrifuged for 30 min at 16,000 g. The supernatant ("crude bromelain extract") was then collected. All operations were carried out at 0-4°C.

Proteolytic activity assays

Proteolytic activity assays followed the method of Pardo *et al*. (2000). The reaction mixture contained 1.1 ml of 1% (w/v) casein solution in 0.1 M glycine sodium hydroxide buffer (pH 8.7) containing 25 mM cysteine and 0.1 ml of crude bromelain extract. The mixture was incubated for 10 min at 37°C; the reaction was stopped by the addition of 1.8 ml of 5% (w/v) trichloroacetic acid (TCA). Blanks were prepared by adding TCA to the crude enzyme extract and then adding the substrate. The mixture was filtered through filter paper (Whatman No. 1). The absorbance of the filtrate was measured at 280 nm. Casein digestive units (CDU) were used to express proteolytic

activity. One CDU was defined as the amount of enzyme that liberated the equivalent of 1 μg of tyrosine in 1 min at 37°C.

Thermal stability

Pineapple juice (6 ml in each test tube) was incubated at different temperatures ranging from 40 to 80°C for 0, 8, 12 and 60 min. Test tubes were quenched cool at 4°C. Crude bromelain extracts were prepared from these cooled pineapple juices, as mentioned above. Residual bromelain activity was measured by the above assay method.

The kinetics of thermal inactivation of the pineapple juice was studied. The inactivation rate constants (k) were calculated from a semi-logarithmic plot of residual activity as a function of time. Activation energy (Ea) for thermal inactivation was calculated from the slope of the Arrhenius plot according to Equation 1 (Whitaker, 1996):

$$\log k = -E\text{a}/(2.303RT) \quad (1)$$

where: k = rate of inactivation at T,
R = gas constant (8.314 J mol^{-1} K^{-1}) and
T = absolute temperature in Kelvin.

Statistical analysis

Linear regression analysis was applied to determine the relationship between log (%residual activity) and time at different temperatures.

RESULTS

Figure 1 shows no fruit bromelain activity loss was observed when fruit bromelain was incubated at 40°C during a period up to 60 min, whereas at 50°C, almost 83% of activity remained. Fruit bromelain activity was retained at 51% after 8 min at 60°C. However, the enzyme was almost completely inactivated by heating for 8 min at 80°C.

The effect of temperature on the stability of an enzyme can be determined. From the log-linear plots of residual fruit bromelain activity against inactivation time at constant temperature, it can be concluded that the thermal inactivation of the enzyme in the temperature range of 40-80° C can be described by a first-order model. Typical plots of data are shown in Figure 2. The enzyme was stable at 40°C, but above 40°C, there was loss of activity, with the higher the temperature, the greater the rate of activity loss.

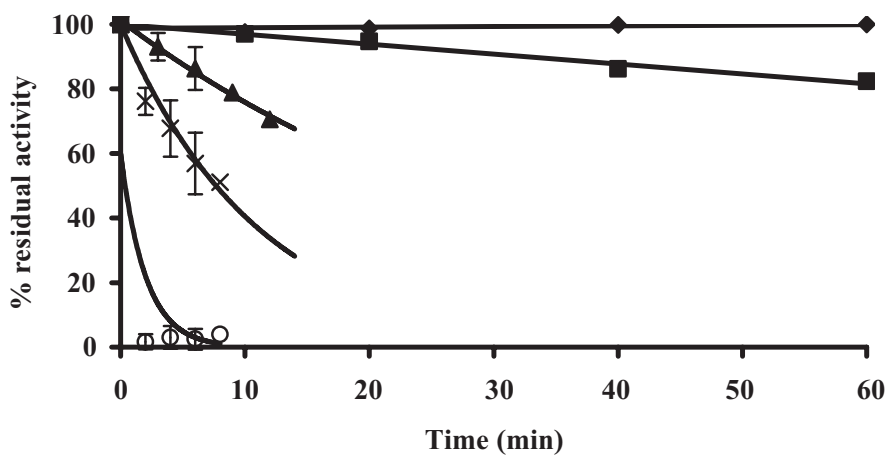

Figure 1 Effect of incubation temperature on fruit bromelain activity: 40(♦), 50(■), 55(▲), 60 (×) and 80°C (○). Fruit bromelain activity was assayed at 37°C with casein as the substrate.

Using the above results, Figure 2 shows the plot of the experimentally determined product concentration versus time at various temperatures. The first order rate constant of the denaturation of enzyme at 40, 50, 55 and 60°C was 0.0001, 0,00145, 0.01255 and 0,03610 min^{-1}, respectively. Figure 3 shows a plot of Log k, the reaction rate constant, versus 1/T. The calculated *E*a value for fruit bromelain inactivation was 313.18 ± 57.44 kJ/mol.

DISCUSSION

From the current study and a previous study by Liang *et al*. (1999), it seems that bromelain from pineapple juice or fruit is more stable when undergoing heating than commercial bromelain obtained from pineapple stems. The current study indicated that bromelain from pineapple fruit retained approximately 51% activity after 8 min at 60°C, and Liang *et al*. (1999)

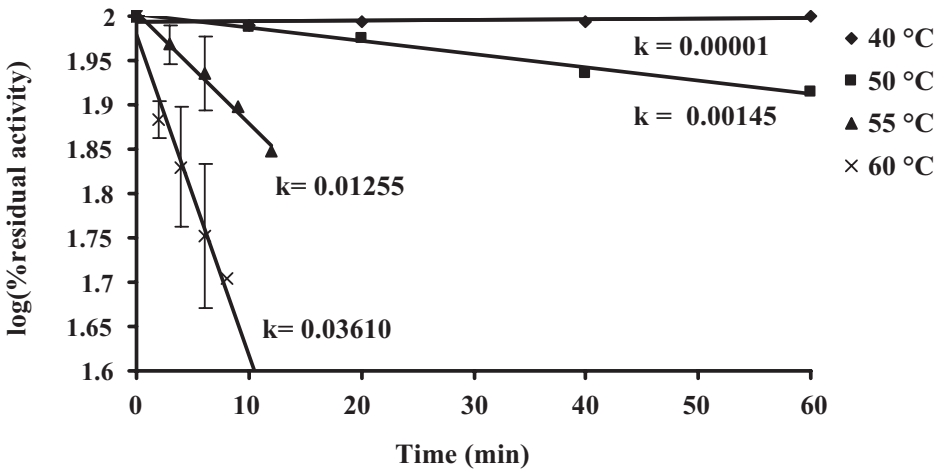

Figure 2 Heat inactivation plots of fruit bromelain at different temperatures. The rate constants (*k*) for inactivation were determined from the slopes of the logarithmic plot of activity against time: log (%residual activity) = -(*k*/2.303)*t*.

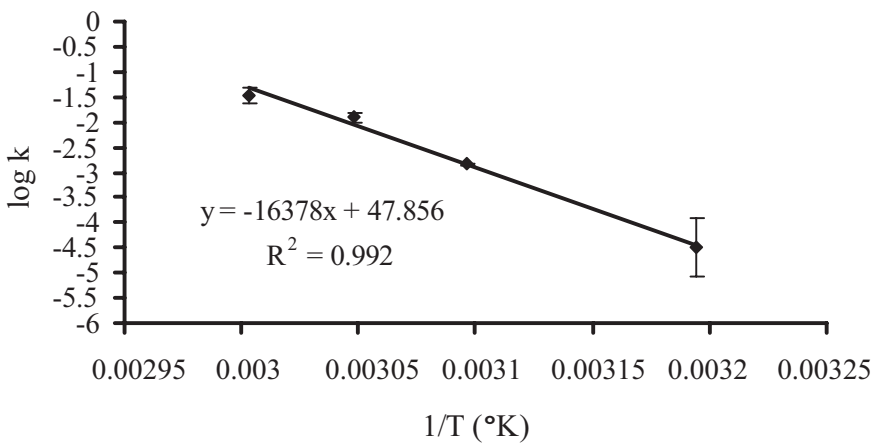

Figure 3 Arrhenius plot for the thermal denaturation of fruit bromelain.

found that bromelain from concentrated pineapple juice remained at 50% activity after 60 min at 60° C. The difference in these results might have been due to differences in the concentrated pineapple juice and the proteolytic method used in these experiments. However, Yoshioka *et al*. (1991) found that commercial bromelain from pineapple stems was completely inactivated by heating for 30 minutes at 60°C, while Gupta *et al*. (2007) found that bromelain retained 50% activity after 20 min at 60°C.

In general, activation energies for the transformation of reactants to products (catalysis) in enzyme-catalyzed reactions are within the range 25.1-62.8 kJ/mol, while activation energies for the denaturation of enzymes are within the range 209.2-627.5 kJ/mol. From a practical standpoint, this means that at lower temperatures, enzymes will be relatively stable. However, at higher temperatures, denaturation will become very rapid, because relatively larger numbers of molecules have sufficient energy to achieve the denatured state (Whitaker, 1994).

The calculated *E*a value for fruit bromelain inactivation in the current study was 313.18 ± 57.44 kJ/mol. This result correlated with the experiment by Sriwatanapongse *et al*. (2000), which found that the *E*a value of bromelain in pineapple juice was 326 kJ/mol. The difference between the value from the current study and Sriwatanapongse *et al*. (2000) was due to differences in the proteolytic activity assay used and the part of the fruit in the pineapple used. Sriwatanapongse *et al*. (2000) used pineapple juice from the flesh and core parts, while the current study used only the fleshy part. Comparable activation energies of commercial pure bromelain extracted from pineapple stems have been reported by Yoshioka *et al*. (1991) (174.47 kJ/mol) and Arroyo-Reyna and Hernandez-Arana (1995) (181 ± 35 kJ/mol). The higher *E*a values of fruit bromelain from pineapple juice also confirmed that it is more stable at high temperature than bromelain from pineapple stems.

CONCLUSION

Temperature stability profiles as a function of different time intervals showed higher retention of enzyme activity at low temperature. Incubation at 40°C showed no fruit bromelain activity loss for up to 60 min, while at 50°C almost 83% of activity remained. Incubation at 80°C for 8 min caused almost complete activity loss. The results from the current study indicated that incubation of pineapple juice at 60°C for 8 min could still allow retention of about 51% of fruit bromelain activity, with corresponding potential health benefits. However, product safety, appearance and taste should ideally be studied side by side. Thermal inactivation of fruit bromelain in the temperature range 40-80°C can be described by a first-order model. The calculated *E*a value for fruit bromelain was 313.18 ± 57.44 kJ/mol.

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