

Title Crude fruit-bromelain affinity to different protein substrates  
Author K. Walizewski and C. Corzo  
Citation Book of Abstracts, 2004 IFT (Institute of Food Technologists) Annual Meeting and Food Expo, 13-16 July 2004, Las Vegas, Nevada, USA. 321 pages.  
Keyword pineapple; bromelain

### Abstract

Pineapple fruit contains proteolytic activity (bromelain) distinct from stem pineapple bromelain. Fruit-bromelain was discovered much earlier than stem-bromelain but much more studies were conducted on stem-bromelain. Crude stem-bromelain is widely used in industry and medicine, but fruit-bromelain is not commercially available, even it can be easily obtained from pineapple juice industry by simple ultrafiltration. The purpose of this study was to characterize proteolytic activity of crude fruit-bromelain using five different substrates: azocasein (pH 3-10 and T=20-70 °C), azoalbumin (pH 4-10 and T=20-65 °C), hemoglobin (pH 2-6.5 and T=30-60 °C), sodium caseinate (pH 2-10 and T=20-70 °C), and casein (pH 2-10 and T=20-70 °C). Fourth grade mature pineapples were peeled and minced in a juice extractor and juice was filtered by a paper filter, centrifuged (20 min, 4 °C and 20,000g) and stored before use at 4 °C. Enzymatic activity was determined in wide range of pH and temperature and reaction was stopped by adding trichloroacetic acid and reaction product was measured at 440 nm (azocasein and azoalbumin), and at 280 for other substrates. Results of  $K_m$  and  $V_{max}$  were calculated by Enzyme kinetics computer program. The most adequate conditions for fruit-bromelain determination are following for: azocasein (pH 6.5, 50 °C,  $K_m$  0.1483 mM,  $V_{max}$  3.85 mmol/min/mg protein), azoalbumin (pH 7.5, 55 °C,  $K_m$  0.079 mM,  $V_{max}$  2.5 mmol/min/mg protein), hemoglobin (pH 2.8-3.5, 37.5 °C  $K_m$  0.322 mM,  $V_{max}$  1.72 mmol/min/mg protein), sodium caseinate (pH 6.5, 60 °C,  $K_m$  0.1 mM,  $V_{max}$  0.972 mmol/min/mg protein) and for casein (pH 7.7, 50 °C,  $K_{max}$  0.147 mM,  $V_{max}$  1.68 mmol/min/mg protein).