High Pressure Spectroscopy (HPS) and Real-time Enzyme Kinetics:

III. Effect of High Pressure and Temperature on Papainase Digestion Rate

1. INTRODUCTION

Papainase is a cysteine protease derived from *Carica papaya* and is commonly used in the food industry as a meat tenderizer. In biotechnology, the enzyme is frequently used for the limited proteolysis of IgG to generate specific Fab and Fc fragments. Medicinally, papainase has been used to liquify the contents of herniated vertebral discs so that their contents can be removed by aspiration [1], in wound debridement [2], and for the removal of dental caries [3] and exhibits antihelmintic properties [4].

High hydrostatic pressure (HPP) has been used for decades in the food industry for the inactivation of microbes and of the oxidases responsible for the browning of fruits and vegetables [5]. In biotechnology, HHP has been shown to accelerate the activity of proteases used for the digestion of proteins prior to mass spectrometry. While the positive effects of temperature and pressure on the activity of several proteases are known [6,7], the potential synergy of elevated temperature with pressure has not been fully characterized, particularly in terms of real-time kinetic studies. Using a high pressure optical cell coupled to a programmable high pressure generator, the effects of elevated pressure (0-60,000 psi) and temperature (22°C to 60°C) on the rate at which papainase digests a synthetic substrate was investigated.

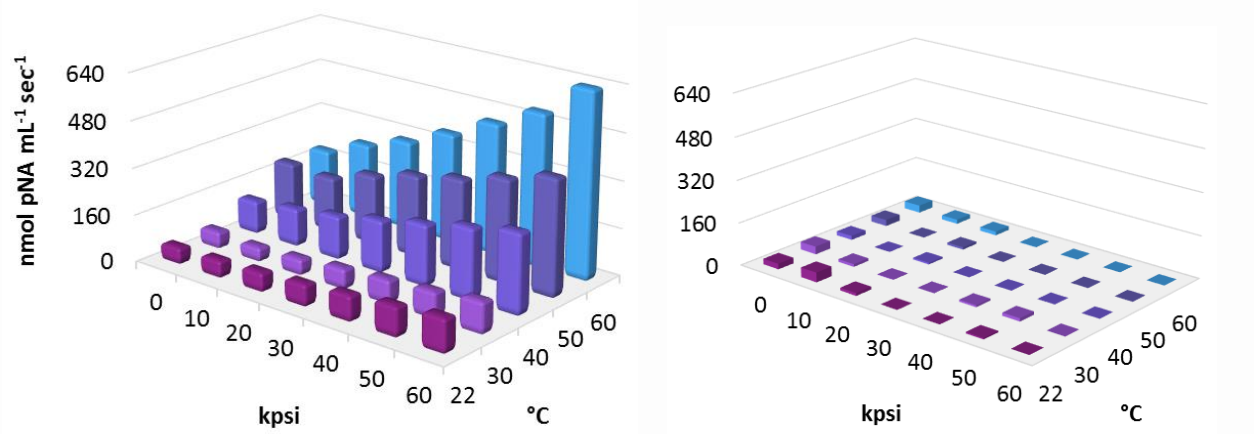

Figure 1. *(A) Papainase digestion of BAPNA at 0-60 kpsi and 22-60°C. (B) Control experiment measuring BAPNA conversion in the absence of enzyme.*

2. METHODS AND MATERIALS

2.1 Papainase

Purified papainase (Sigma-Millipore, Burlington, MA, USA) was reconstituted to 9.3 mg/mL concentration in water. The substrate N-benzoyl-L-arginine p-nitroanilide (BAPNA) was prepared as a 115 mM stock solution in DMSO. Papainase was diluted to 40 ug/mL in 100 mM potassium phosphate pH 6.4 reaction buffer containing 5 mM L-cysteine and 2.3 mM BAPNA.

2.2 High pressure spectroscopy

High pressure spectroscopy was performed in a high pressure optical cell equipped with 6.4 mm thick sapphire windows (ISS, Champaign, IL, USA). The optical cell was interfaced with a UV-2501PC UV/Vis recording spectrophotometer (Shimadzu, Kyoto, Japan) using the UVProbe 2.5 software for data acquisition. The optical cell was pressurized sequentially to 10, 20, 30, 40, 50 and 60 kpsi in a HUB880 pressure generator (**Figure 2**). The optical cell was pressurized and depressurized at a rate not exceeding 1000 psi/sec. Pressure was held constant for 180 seconds at each pressure, as programmed using the ramp mode feature of the HUB Version 2.3.6 software, during which time absorbance at 405 nm was recorded at one second intervals.

The optical cell was heated with four 45W cartridge heaters (McMaster-Carr, Princeton, NJ, USA) controlled by a PBI Intelligent Temperature Controller (PBI, South Easton, MA, USA) with thermocouple interface. Temperature of the optical cell was maintained to within 1.4°C of the target temperature.

A. **B.**

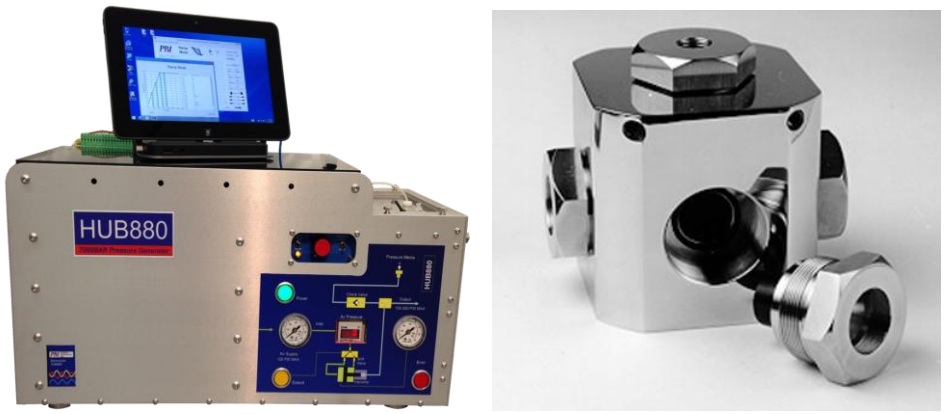

Figure 2. ***(A)*** *The HUB880 programmable high pressure generator and* ***(B)*** *high pressure optical cell used for real-time HPS.*

3. RESULTS AND DISCUSSION

The rate of papainase conversion of the synthetic substrate BAPNA measured by real time HPS was accelerated as a function of pressure at all temperatures tested (Figure 1). At room temperature, papainase activity was increased over 2.2 times at 60 kpsi compared to atmospheric pressure. This suggests that papainase digestion of foods could be effectively performed at higher pressures while maintaining more desirable low temperatures. The highest rate observed was at 60 kpsi and 60°C. Further investigation is required to determine the pressure and temperature maxima for this enzyme.

4. REFERENCES

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