Electronic Supplementary Information (ESI)

Effect of proteolysis on degradation of Pru p 3

Results showed that its activity decreased markedly with the decrease of pH to 6.0 and 5.0, being necessary very long times (72 h) at 25 °C to achieve a considerable degradation.

S1. SDS-PAGE

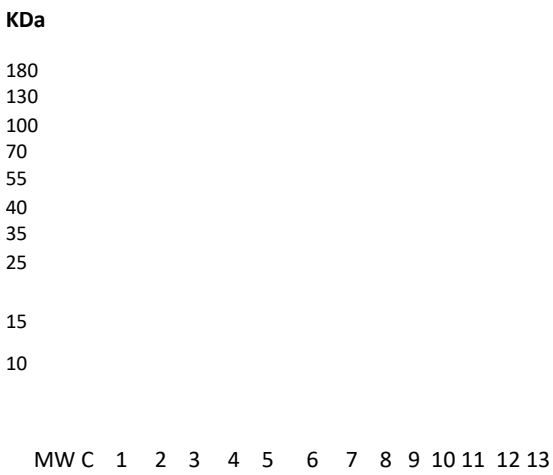

Figure S1. SDS-PAGE on polyacrylamide gel (4–20%) under reducing conditions of Pru p 3 treated with protease from bovine pancreas under different conditions. The pH, temperature (°C) and time (h) of treatment are indicated in parentheses. Molecular weight marker (MW). Control untreated Pru p 3 (C). Lane 1, (7/25/24). Lane 2, (7/4/24). Lane 3, (6/25/24). Lane 4 (6/4/24), Lane 5, (5/25/24). Lane 6, (5/4/24). Lane 7, (7/25/72). Lane 8, (7/4/72). Lane 9, (6/25/72). Lane 10, (6/4/72). Lane 11, (5/25/72). Lane 12, (5/4/72). Lane 13, (7/37/72).

The analysis of hydrolysates obtained with ASP by MALDI-TOF MS is shown in Fig. S2 a-e. In the hydrolysates generated with ASP, the 9 kDa peak belonging to Pru p 3 was not observed and the predominant peptides obtained had molecular weights of less than 3.2 kDa and 1.8 kDa for treatments at 50 °C for 2 h and 25 °C for 24h, respectively. However, the sample incubated with protease from *Rhizopus* (Fig. S2 d) displays a similar chromatographic profile to that obtained with the native protein, indicating that it is not able to degrade Pru p 3.
The analysis of the ASP in buffer by MALDI-TOF MS gave peaks within the molecular weight range from 20 to 100 kDa, as it was observed in the electrophoretic profile (Fig. S2 e).

S2. MALDI TOF mass spectrum

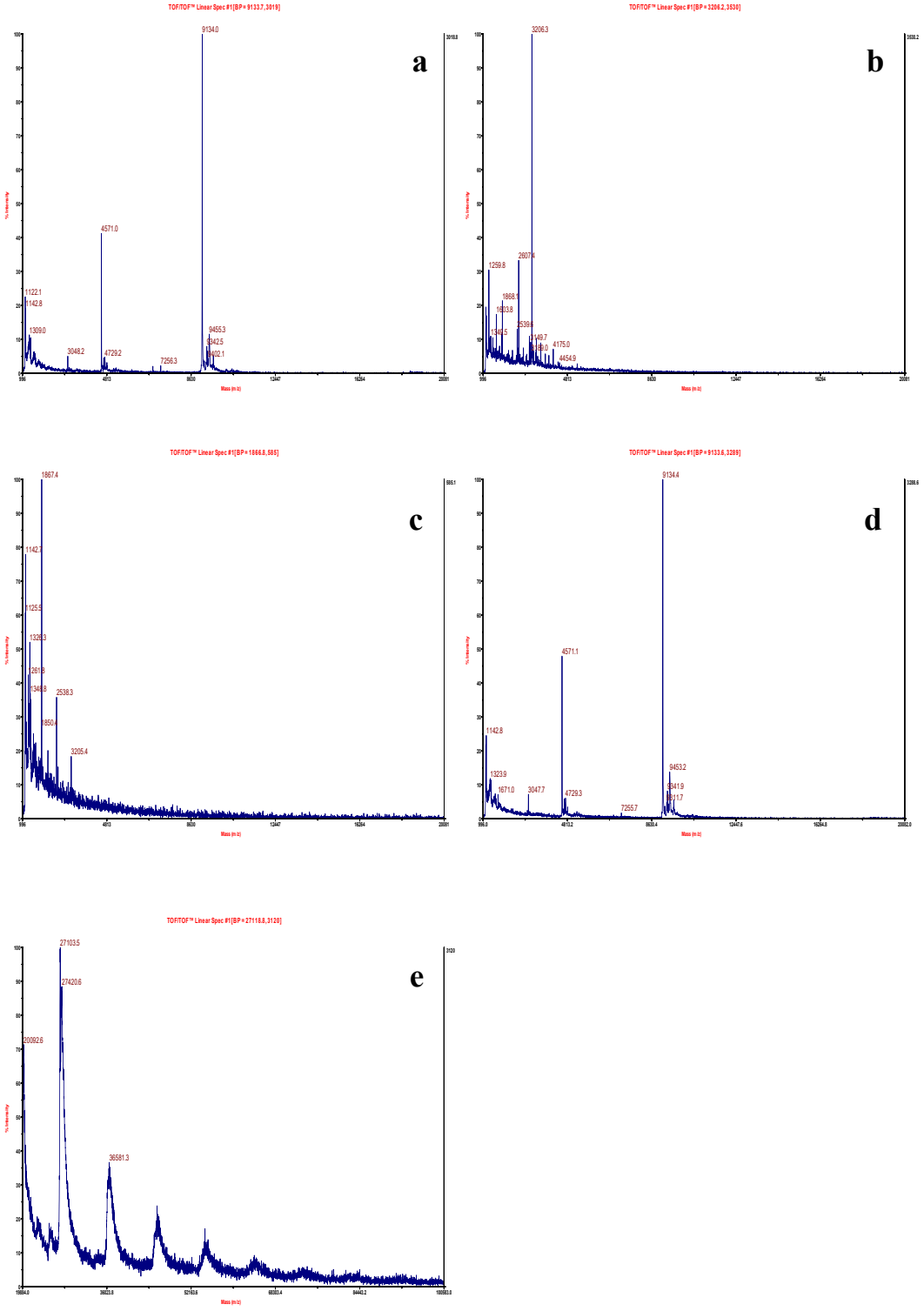

Figure S2. MALDI TOF mass spectrum of native Pru p 3 (a) Pru p 3 incubated with Acid Stable Protease (ASP) at 50°C for 2h (b) or at 25°C/24h (c) or with protease from *Rhizopus* at 50°C for 2h (d) or only Acid Stable Protease (ASP) (e).

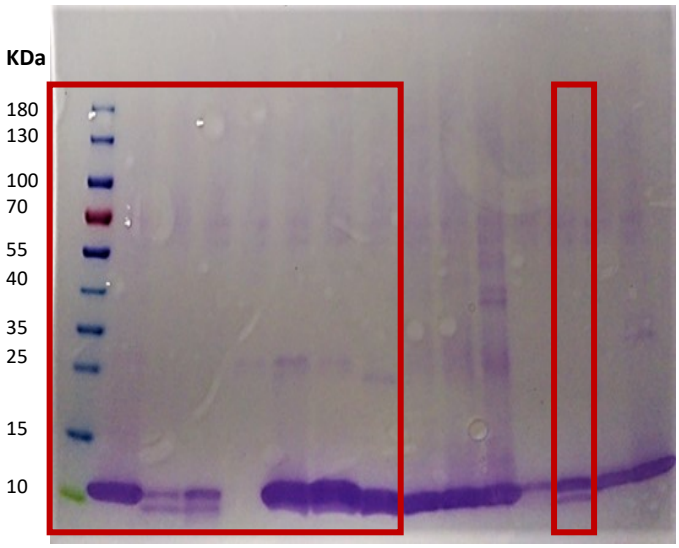

Figure S3: Uncropped gel which corresponds to the image of Figure 1 (only the lanes 1-8 and 13)

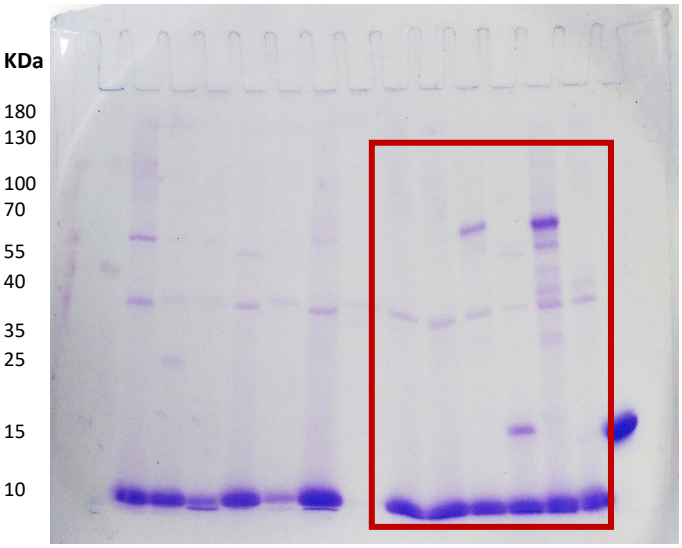

Figure S4: Uncropped gel which corresponds to the image of Figure 1 (only the lanes 9-14)

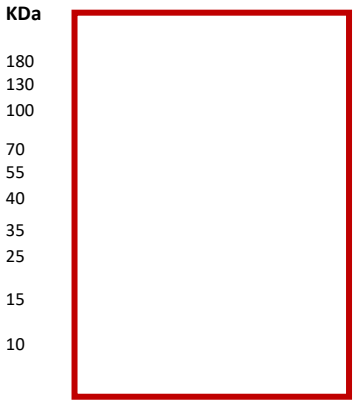

Figure S5: Uncropped gel which corresponds to the image of Figure 1 (only the lanes 1-7)