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Biochemical characteristics of the extracellular protease from *Pediococcus pentosaceus* isolated from Harbin dry sausages (#343)

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Introduction

Harbin dry sausage is a traditional Chinese natural fermented meat product which has a short production cycle, a unique local taste, fine texture and good flavour. *Pediococcus pentosaceus* has been identified in Harbin dry sausage, the aim of this research was to identify the protease purified from *P. pentosaceus* and to characterize the factors affecting protease activity, and study the potential possibilities of microbial protease to give the Harbin dry sausages unique physicochemical and sensorial properties originating from its specific activity.

Methods

1. Protease preparation

P. pentosaceus was isolated from Harbin dry sausage, extracellular protease was purified using ammonium sulphate deposition, ion exchange layer system and gel filtration, The active protease collections were quickly freeze-dried and stored at -20 °C for further investigation. The protease activity was determined with 2% casein substrate solution in a 0.02 M phosphate buffer (pH 6.8) at 37 °C.

2. Effect of pH and temperature on activity and stability of the protease

The protease was dissolved for different pH and the mixture was incubated for 0-60 min, and then the relative protease activity was measured sequentially for each pH and time. The optimal temperature was determined by measuring the protease activity using a 2% casein solution as the substrate at pH 6, and varying the temperature from 20 to 60 °C, 0-60 min. The relative protease activity was measured sequentially for each temperature and time.

3. Effect of metal ions and inhibitors on the protease activity

Different metallic ions or inhibitors at 1 mmol/L and 10 mmol/L were used to examine their effect on the protease activity. Then, protease with a protein concentration of 10 mg/mL (1 mL) was incubated with a solution (1 mL) of chloride salts of each ion or protease inhibitors at 37 °C for 30 min, the protease activity in the absence of any ions or inhibitors was defined as 100% (control).

4. Efficacy of protease in hydrolysing myofibrillar and sarcoplasmic protein

The concentrations of myofibrillar and sarcoplasmic proteins were adjusted to 3.0 mg/mL, and 3 mL of the two meat proteins were collected in a tube, then the protease (40 U/g) was added. The time evolution samples of the hydrolysis assay were obtained by retrieving aliquots (100 μL) from the tube after 10-70 min, and the hydrolysis evolution with time was evaluated by SDS-PAGE.

Results

1. Effect of pH and temperature on activity and stability of the protease

Fig. 1A shows that the purified extracellular protease exhibited a maximum level of relative activity (100%) at pH 6. The stability of the purified protease in all pH environments showed a similar variation trend during the entire incubation time, maintaining the relative activity above 50% during 25 min of incubation, and the purified protease exhibited a relatively good pH stability at pH 6 (Fig. 1B).

Fig. 1C reflects the optimal temperature was observed to be approximately 30 °C and the relative activity (100%) was significantly higher than that at the other temperatures ($P<0.05$). As shown in Fig. 1D, the thermal stability and the relative protease activity were poor at 50 °C and lower than 50% at 30 min, while the relative activity at 30 °C was greater than 65% at 30 min.

2. Effect of metal ions and inhibitors on the protease activity

The effect of various metal ions on *P. pentosaceus* protease relative activity was investigated at 1 mmol/L and 10 mmol/L (Table 1). K^+ and Ca^{2+} had no significant effect at 1 mmol/L ($P>0.05$) and stimulated the protease activity up to 105.8% and 113.2% at 10 mmol/L ($P<0.05$). Compared with the control, the protease relative activity in the presence of 1 mmol/L and 10 mmol/L Cu^{2+} was significant reduced to 45.7% and 20.4%, respectively ($P<0.05$). The protease activity decreased to 67.6% and 21.9% in the presence of 1 mmol/L and 10 mmol/L EDTA , and decreased to 92.4% and 91.3% in the presence of 1 mmol/L and 10 mmol/L PMSF, respectively ($P<0.05$).

3. Efficacy of protease in hydrolysing myofibrillar and sarcoplasmic protein

Hydrolysis of meat myofibrillar and sarcoplasmic proteins has been considered as the source of the fermented meat flavour compounds. The *P. pentosaceus* protease exhibited a certain degree of enzymatic activity on myofibrillar protein (Fig. 2A). With the extension of the action time from 0 min to 70 min, the bands intensity corresponding to 220, 97.2, 44.3, and 40 kDa vanished or decreased while degradation products with low molecular weights ranging from 14.3 to 40.0 kDa simultaneously appeared. The *P. pentosaceus* protease showed an extremely high enzymatic activity and excellent reaction rate for sarcoplasmic protein (Fig. 2B). The intensities of the sarcoplasmic protein bands (29-220 kDa) gradually decreased with longer reaction time.

Notes

Conclusion

P. pentosaceus protease reached higher relative protease activity at pH 6 and 30 °C, and the protease showed certain pH and thermal stability at pH 6 and 30 °C. *P. pentosaceus* protease activity can be inhibited by 1 mmol/mL and 10 mmol/mL EDTA. SDS-PAGE experiments show the ability of protease to hydrolyse sarcoplasmic and myofibrillar proteins.

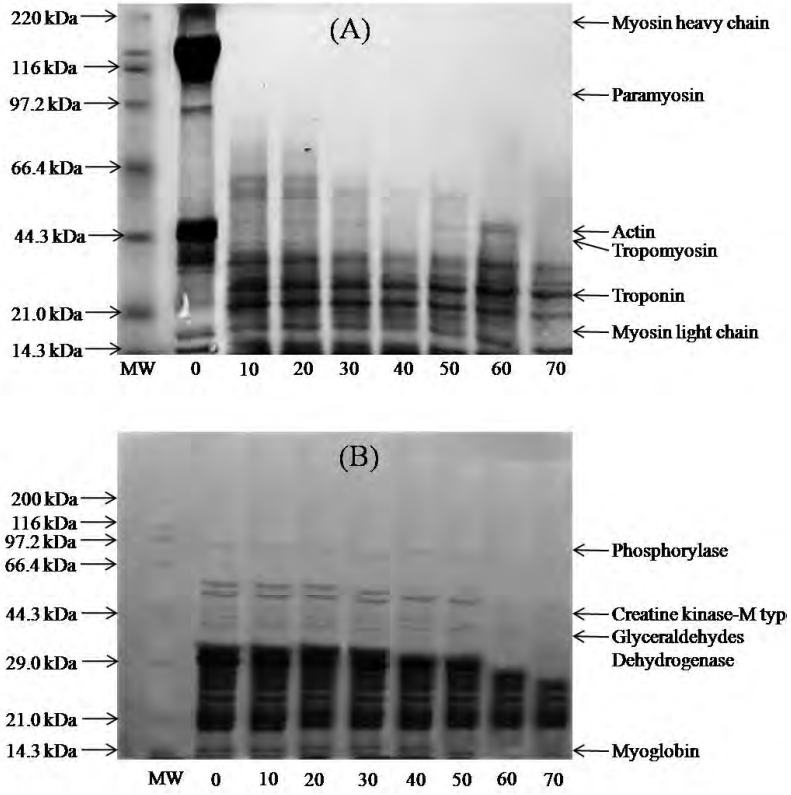

Fig. 2. Efficacy of protease in hydrolysing myofibrillar and sarcoplasmic protein Enzymatic hydrolysis of myofibrillar (A) and sarcoplasmic (B) proteins by *P. pentosaceus* purified protease. MW refers to the molecular weight of the protein standard; 0-70 refers to the time of enzymolysis (min).

Compounds	1 mmol/L	10 mmol/L
None	100^{ab}	100^{cd}
Na^{+}	102.7 ± 1.5^{a}	104.6 ± 1.3^{bc}
K^{+}	103.6 ± 3.2^{a}	105.8 ± 1.0^{b}
Mg^{2+}	97.4 ± 1.2^{b}	76.1 ± 2.2^{f}
Ca^{2+}	101.9 ± 1.4^{ab}	113.2 ± 3.9^{a}
Mn^{2+}	86.5 ± 1.4^{d}	64.9 ± 1.4^{g}
Zn^{2+}	82.2 ± 1.1^{d}	70.0 ± 2.0^{g}
Cu^{2+}	45.7 ± 1.6^{f}	20.4 ± 1.8^{i}
Fe^{2+}	92.0 ± 1.2^{c}	95.1 ± 1.9^{de}
Fe^{3+}	82.2 ± 1.8^{d}	36.8 ± 1.4^{h}
EDTA	67.6 ± 1.7^{e}	21.9 ± 0.5^{i}
PMSF	92.4 ± 1.4^{c}	91.3 ± 1.1^{e}

Table 1 Effects of different concentrations of metal ions and inhibitors on the protease activity Purified protease was incubated with various reagents at 37°C for 30 min, and 100% relative activity was assigned to the activity in the absence of reagents. $^{a-i}$ Means values (mean ± standard error) within the same column with different uppercase letters differ significantly from each other ($P < 0.05$).

Notes

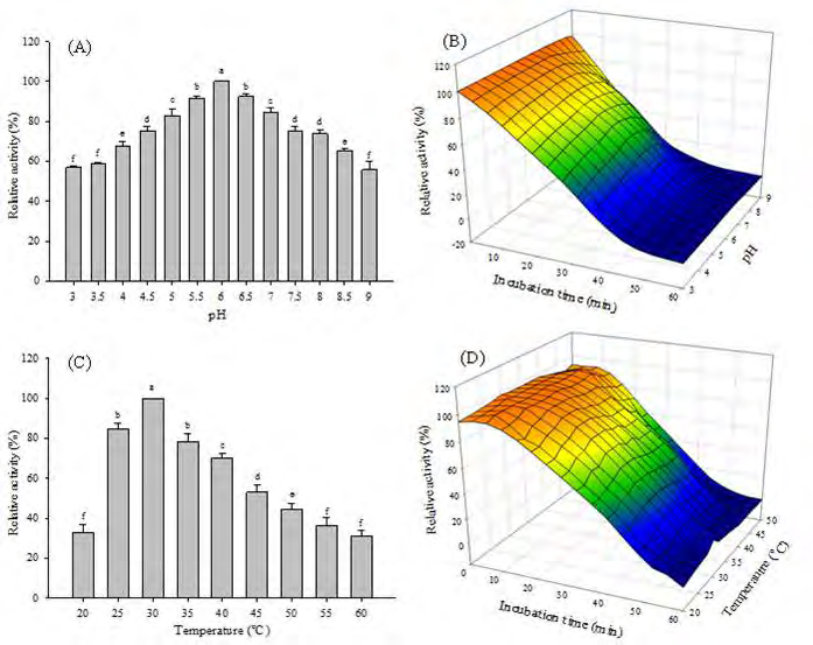

Fig. 1. Effect of pH and temperature on activity and stability of the protease

Effect of pH on the protease activity (A) and stability (B), the influence of the temperature on protease activity (C) and stability (D). Error bars refer to the standard error obtained from the analysis of triplicate samples. Different letters (a-f) indicate the significant differences in the crude protease activity at different initial pH and fermentation temperatures ($P < 0.05$).

Notes