Fungal and Bacterial Proteases: Characteristics, and Opportunities for the Processing of Plant Proteins

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Introduction

Hydrolysis of proteins improves their functional and bioactive properties.

Fungal and bacterial proteases have been used to hydrolyse a wide range of animal substrates[1].

This work aimed to:

1. Screen a range of bacterial and fungal proteases.
2. Evaluate the effects of selected bacterial and fungal proteases on a plant protein substrate.

Methods

Enzyme screening: Six commercially available proprietary fungal (FPII, F31K, F60K), bacterial (HT, 4000 P, BS Conc), and plant (papain) proteases.

- **Casein hydrolysis activity[2]-**

1. Enzyme suspended in deionized water (1 mg/mL), centrifuged and diluted as required.
2. Enzyme solution (50 µL) + 50 µL BODIPY-FL substrate + 50 µL buffer (sodium phosphate, pH 6.8).
3. Change in fluorescence measured over 10 min at 40 °C (excitation 485 nm, emission 520 nm).

- **Soluble protein of enzymes-** Bradford method.

Protein hydrolysis: HT and F31K were selected and used to hydrolyse a commercial Brown Rice Protein Powder (80% protein).

Brown Rice Protein Powder suspended in phosphate buffer (pH 6) at 1:3 (Powder:Buffer (W/V))

pH adjusted to optimise hydrolysis

HT: pH 6 | F31K: pH 8.5

Protease powder added at 1% of Brown Rice Protein concentration

Hydrolysis for up to 120 min

HT: 65 °C | **F31K:** 45 °C

- **Soluble protein in hydrolysates-** Bradford method and SDS-PAGE.

Results

Enzyme screening

Table 1: Casein Hydrolysis Activity (Δfluo/min/mg soluble protein) of selected proprietary fungal, bacterial, and plant proteases (Mean ± SD)*

Enzyme	Source	Casein Hydrolysis Activity
HT	Bacterial	$3.12\times10^4 \pm 4.09\times10^2$ f
4000 P		$39.5\times10^4 \pm 151\times10^2$ a
BS CONC		$17.0\times10^4 \pm 206\times10^2$ c
FPII	Fungal	$3.55\times10^4 \pm 10.6\times10^2$ ef
F31K		$21.5\times10^4 \pm 113\times10^2$ b
F60K		$4.25\times10^4 \pm 66.5\times10^2$ e
Papain	Plant	$7.11\times10^4 \pm 41.3\times10^2$ d

* Superscript letters indicate significant differences between proteases using ANOVA and Tukey's Test ($p < 0.05$).

Protein hydrolysis

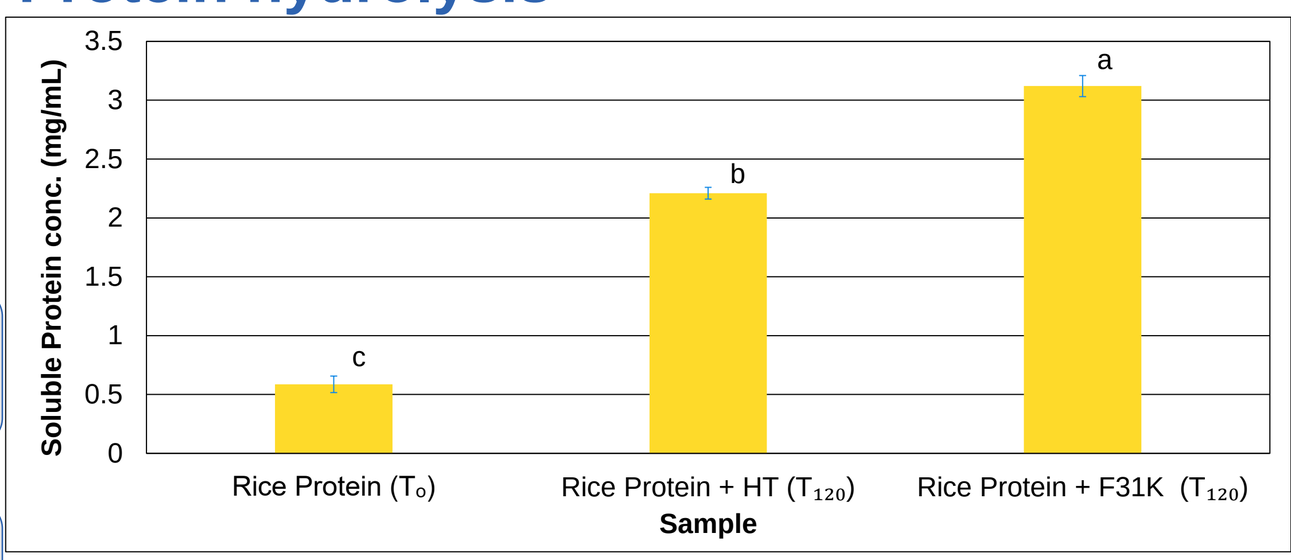

Fig 2: Soluble protein conc. of Brow Rice Protein Powder before and after Hydrolysis for 120 min (Mean ± SD), Superscript letters indicate significant differences between proteases using ANOVA and Tukey's Test ($p < 0.05$).

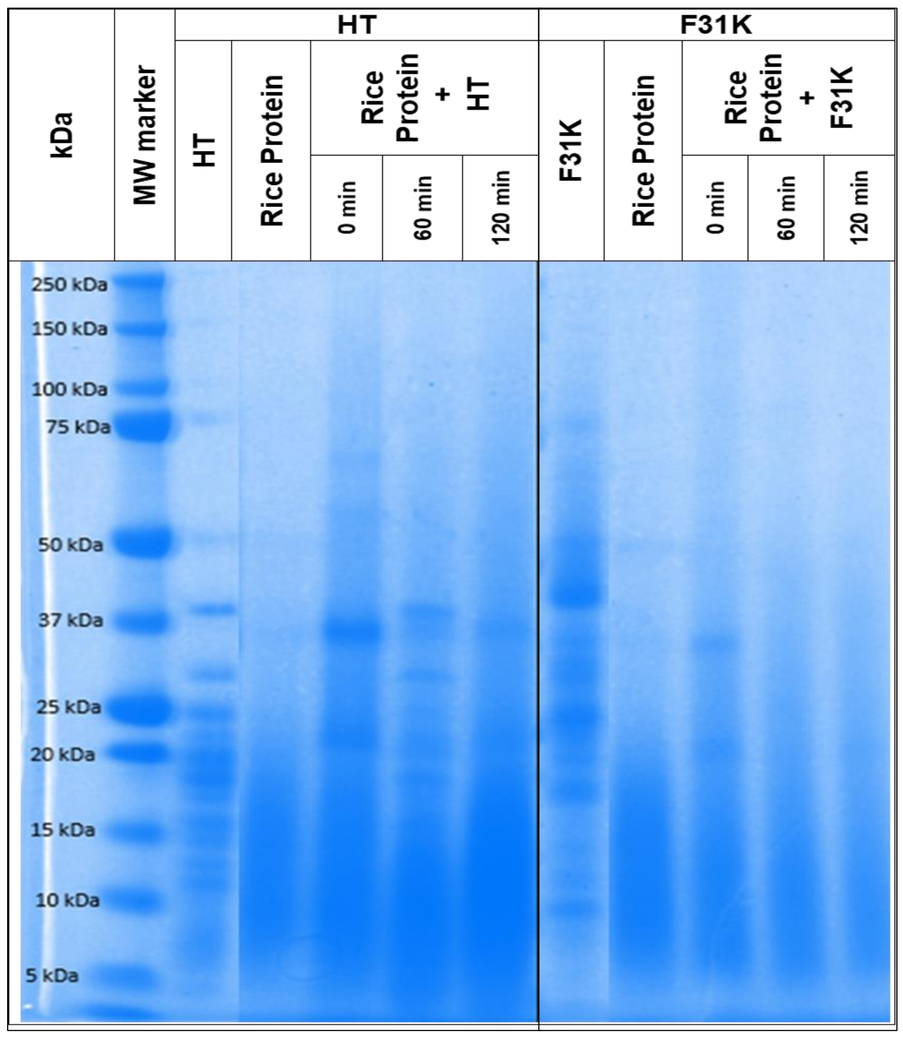

Fig 1: Qualitative SDS-PAGE of the soluble fraction of Brown Rice Protein Powder before and after Hydrolysis.

Conclusions

Fungal and Bacterial protases show a wide range of hydrolysis activities giving them great potential for applications in a wide range of food products.

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References

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2. Thompson, V. F., Saldaña, S., Cong, J., & Goll, D. E. (2000). A BODIPY fluorescent microplate assay for measuring activity of calpains and other proteases. *Analytical Biochemistry*, 279(2), 170–178. https://doi.org/10.1006/abio.1999.4475