Development of Oyster Mushroom-Derived Milk-Clotting Enzymes and Antioxidant-Rich Halal Vegan Cheese through Proteomic Characterization

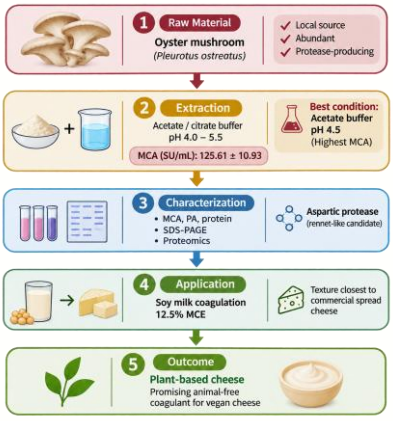
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Abstract

Oyster mushroom (*Pleurotus ostreatus*) was investigated as a sustainable source of milk-clotting enzymes (MCE) for plant-based cheese production. Extraction using acetate and citrate buffers (pH 4.0–5.5) was evaluated for enzyme activity, selectivity, and protein composition. Acetate buffer at pH 4.5 achieved the highest milk-clotting activity (125.61 SU/mL), while refrigerated centrifugation improved enzyme selectivity approximately 2.4-fold. Proteomic analysis identified an aspartic protease as a promising rennet-like candidate. Texture Profile Analysis (TPA) showed that 12.5% MCE produced soy cheese with properties closest to commercial spread cheese. These findings demonstrate the potential of oyster mushroom MCE as an alternative vegan coagulant.



Keyword: Plant-based cheese, Protease, Rennet substitute

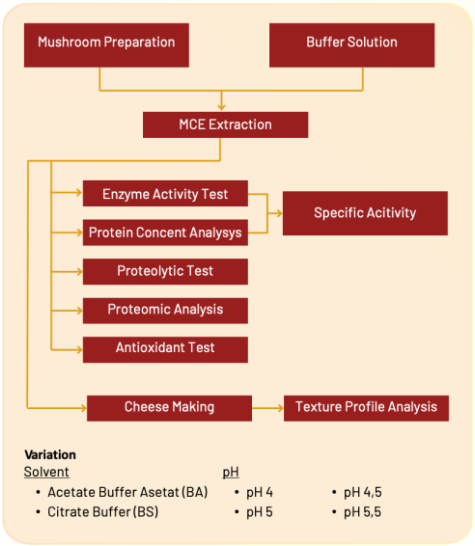
Introduction

Global demand for plant-based foods is rapidly increasing, with the vegan cheese market projected to grow at a **compound annual growth rate (CAGR) of 10.4%** (Plant Based Food Market, 2024). However, the limited supply of conventional animal rennet, meeting only **20–30% of global milk coagulant demand**, highlights the need for alternative coagulants. Oyster mushroom (*Pleurotus ostreatus*) offers a promising source of **milk-clotting enzymes (MCE)** due to its proteolytic activity and abundant local availability, with West Java producing **170,771.04 quintals in 2024**. This study aims to optimize MCE extraction and evaluate its effectiveness in coagulating plant-based milk for vegan cheese production.



Fig 1. Oyster Mushroom

Research Method



Discussion & Result

Proteolytic Acitivity (PA)

The enzyme's ability to hydrolyze proteins randomly and non-specifically

Solvent	pH	Proteolytic Activity (U/mL)
Acetate Buffer	4.0	0.67
	4.5	1.24
	5.0	1.91
	5.5	1.33
Citrate Buffer	4.0	0.36
	4.5	0.53
	5.0	0.38
	5.5	0.49

Acetate buffer exhibited higher proteolytic activity than citrate buffer, with the highest activity observed at pH 5.0.

Protein Content

The amount of crude protein solubilized or extracted into the buffer.

Solvent	pH	Total Protein (mg/mL)
Acetate Buffer	4.0	0.16 ± 0.01
	4.5	0.20 ± 0.02
	5.0	0.30 ± 0.03
	5.5	0.47 ± 0.04
Citrate Buffer	4.0	0.43 ± 0.02
	4.5	0.47 ± 0.03
	5.0	0.51 ± 0.06
	5.5	0.58 ± 0.03

Citrate buffer yielded a higher total protein content than acetate buffer. The highest protein content was observed with citrate buffer at pH 5.5.

Enzyme Activity Tests

Milk-Clotting Enzyme Activity (MCA): *The enzyme's ability to induce milk coagulation.*

Specific Activity: *Enzyme activity per unit of protein, indicating relative enzyme purity.*

MCA/PA Ratio: *Enzyme selectivity for milk clotting over nonspecific protein hydrolysis; higher ratios indicate better selectivity.*

Enzyme Activity Tests Results

Solvent	pH	MCA (SU/mL)	Specific Activity (SU/mg)	MCA/PA Ratio
Acetate Buffer (BA)	4.0	111.57 ± 10.52	714.21 ± 106.46	169.69 ± 7.5
	4.5	125.61 ± 10.93	637.61 ± 102.85	97.76 ± 14.88
	5.0	105.06 ± 9.44	353.72 ± 20.95	54.95 ± 2.11
	5.5	102.50 ± 10.23	221.91 ± 36.8	73.69 ± 3.14
Citrate Buffer (BS)	4.0	88.95 ± 9.72	209.41 ± 33.09	252.64 ± 32.56
	4.5	98.09 ± 7.47	235.06 ± 11.77	215.74 ± 62.45
	5.0	87.56 ± 6.00	181.05 ± 18.63	238.22 ± 50.58
	5.5	79.39 ± 6.34	153.10 ± 3.94	180.70 ± 23.53

Specific Activity & MCA/PA Ratio

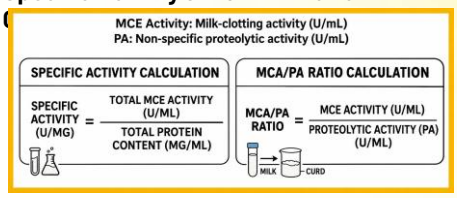




Fig 2. Enzyme activity, proteolytic and TPA tests

- Acetate buffer exhibited higher MCE activity than citrate buffer, **with the highest activity observed at pH 4.5**.
- The highest specific activity was obtained using acetate buffer at **pH 4.0**.
- Citrate buffer exhibited higher MCA/PA ratios, **with the highest ratio observed at pH 5.0**.

Texture Profile Analysis

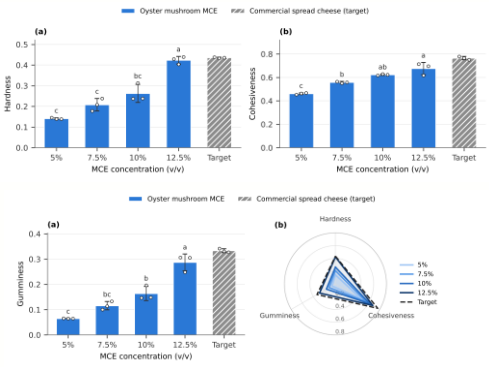


Fig 3. Texture Profile Analysis

Conclusion

- **Optimal Extraction:** Acetate buffer at pH 4.5 achieved the highest milk-clotting activity (125.61 SU/mL).
- **Enzyme Selectivity:** Refrigerated centrifugation improved the MCA/PA ratio by approximately 2.4-fold.
- **Proteomics:** An aspartic protease was identified as a promising rennet-like enzyme candidate.
- **Cheese Texture (TPA):** A 12.5% MCE concentration produced soy cheese with texture closest to commercial spread cheese.
- **Antioxidant Activity:** Crude extracts showed modest activity, but further work needed to retain the activity in the cheese.