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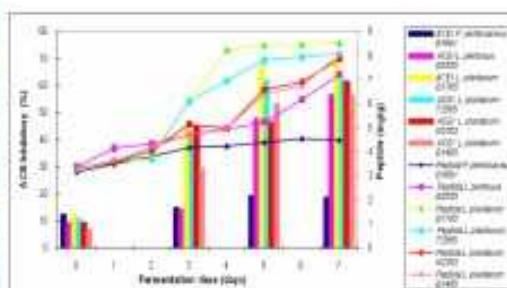
Abstract

Bekasam is an Indonesian traditional fermented fish. Bekasam is perceived to have an angiotensin converting enzyme (ACE) inhibitory activity, which is thought to be the activity of bioactive peptides, the product of protein degradation during the bekasam fermentation. The inhibition of ACE activity correlated with antihypertensive activity. Some strains of lactic acid bacteria that had a proteolytic activity had been isolated from bekasam and were identified belong to species of *Lactobacillus plantarum* B1765, *L. plantarum* T2565, *L. plantarum* N2352, *L. plantarum* B1465, *Lactobacillus pentosus* B2555, and *Pediococcus pentosaceus* B1661. The proteolytic activity was known to be correlated with the production of the ACE inhibitor in bekasam like product and the highest ACE inhibitor activity (67.18%) was produced by *L. plantarum* B1765. This isolate then was used as a starter culture in bekasam fermentation process to be evaluated the improvement of the ACE inhibitor activity. The result showed that *L. plantarum* B1765 increased the ACE inhibitor activity to 71.54%, higher than control (52.99%) which was not inoculated with this isolate. The used of starter culture was also improving the quality product of bekasam by increasing the total lactic acid bacteria, soluble protein, decreasing pH, and shorten the fermentation process.

Key words : ACE inhibitor, bekasam, fermented fish, lactic acid bacteria, proteolytic activity

Result

1. The Potency of The Proteolytic LAB of Bekasam to Produce ACEI



Peptide and ACE Inhibitory of the proteolytic LAB of bekasam

- There was a correlation between the peptide concentration and the ACEI activity produced by LAB of bekasam isolates.
- The highest producing peptide (120 ± 0.36 mg/g) to 3.41 ± 0.02 mg/g was found in *L. plantarum* B1765, and the smallest one (1.26 ± 0.25 mg/g) to 1.77 ± 0.38 mg/g in *P. pentosaceus* B1661.
- L. plantarum* B1765 exhibited the highest ACEI activity (67.17 ± 1.32%), followed by *L. plantarum* T2565 (62.54 ± 2.11%), *L. plantarum* N2352 (61.36 ± 1.22%), *L. plantarum* B1465 (59.85 ± 1.18%), and *L. plantarum* B2555 (56.61 ± 0.28%), whereas *P. pentosaceus* B1661 showed the smallest one (38.66 ± 3.91%).

Introduction

Angiotensin Converting Enzyme Inhibitor (ACEI) correlated with the antihypertensive activity. This activity is showed by the bioactive peptides, the product of protein degradation by endogenous enzyme or microbes enzymes or proteolytic enzyme in vitro (Kitt and Weller, 2003; Yamamoto et al., 2003). Lactic acid bacteria was known had a role in producing of ACEI in some fermented food. Although the antihypertensive mostly were studied on fermented milk, but some researches found that some of fermented fish showed this activity (Gobetti et al., 2000; Seppo et al., 2003; Yamamoto et al., 2003; Yin et al., 2004; Ito and Akahane, 2004; Muguerza et al., 2006; Ito et al., 2007).

Bekasam, a traditional Indonesian fermented fish, was known has ACEI activity, which is thought to be the activity of bioactive peptides, the product of proteolytic lactic acid bacteria (LAB) during the bekasam fermentation (Wikandari et al., 2009; Wikandari et al., 2011).

We had isolated some strains of LAB that had a proteolytic activity from bekasam and were identified belong to species of *Lactobacillus plantarum* B1765, *L. plantarum* T2565, *L. plantarum* N2352, *L. plantarum* B1465, *L. pentosus* B2555, and *Pediococcus pentosaceus* B1661 (Wikandari et al., 2010).

Objective

- To evaluate the potency of the proteolytic LAB of bekasam isolates to produce ACEI.
- To evaluate the potency of the ACEI-producing lactic acid bacteria as a starter culture.

in bekasam fermentation to improve the ACEI activity and the quality of bekasam.

Material & Methods

Material

Milkfish from local market, *Lactobacillus plantarum* B1765, *L. plantarum* T2565, *L. plantarum* N2352, *L. plantarum* B1465, *L. pentosus* B2555, and *Pediococcus pentosaceus* B1661 isolated from bekasam, MRS growth medium, OPA, Lys-Gly, ACE from rabbit lung, Hip-His-Leu, etc.

Methods

- Screening of ACEI-producing LAB
- Milk fish was gutted, eviscerated, deboned, washed and then mixed with 10% NaCl and rice 1:1, sterilized for 30 minutes at 115°C. The mixture then inoculated with 10% CFU/g of the tested isolate and fermented for 7 days.
- Fermentation of bekasam by selected ACEI-producing LAB as starter culture.
- Milk fish was gutted, eviscerated, deboned, washed and then mixed with 10% NaCl, fermented for 2 days, and then inoculated with 10% CFU/g of the selected ACEI-producing lactic acid bacteria isolates and fermented for 7 days. No inoculated sample was used as a control.
- Parameter test: ACEI activity (Chauman and Chung,), peptide (OPA method), the number of LAB (SPC method), pH and total acid (AOAC,).

2. The Role of the *L. plantarum* B1765 As a Starter Culture in Improvement The Quality And The ACEI Activity of Bekasam.

Table of The Quality and The ACEI Activities of Bekasam Fermented by *L. plantarum* B1765 As A Starter Culture

Fermentation time (days)	Number of LAB (CFU/g)		pH		Peptide (mg/g)		ACEI (%)	
	No. starter	Starter Culture	No. starter	Starter Culture	No. starter	Starter Culture	No. starter	Starter Culture
0	4.86 ± 0.51 10 ⁷ ± 1.48	5.31 10 ⁷ ± 1.48	6.27	6.25	3.63	3.51	3.39	3.50
1	4.91 ± 0.86 10 ⁷ ± 0.96	4.86 10 ⁷ ± 0.96	6.27	6.25	3.49	3.58	-	-
2	4.73 ± 0.45 10 ⁷ ± 0.34	4.73 10 ⁷ ± 0.34	5.76	5.74	3.73	4.03	-	-
3	7.42 ± 0.40 10 ⁷ ± 1.10	7.42 10 ⁷ ± 1.10	5.42	5.45	5.46	4.72	4.43	5.18
4	6.23 ± 0.18 10 ⁷ ± 0.27	6.18 10 ⁷ ± 0.27	5.05	4.99	6.71	5.76	-	-
5	5.70 ± 0.10 10 ⁷ ± 0.06	6.10 10 ⁷ ± 0.10	4.52	4.56	7.03	7.07	41.35	72.54
6	1.30 ± 0.02 10 ⁷ ± 0.20	1.18 10 ⁷ ± 0.20	4.41	4.37	7.03	6.61	-	-
7	1.37 ± 0.10 10 ⁷ ± 0.20	1.10 10 ⁷ ± 0.20	4.28	4.18	7.25	10.10	45.99	68.08
8	1.46 ± 0.20 10 ⁷ ± 1.00	8.10 10 ⁷ ± 1.00	4.21	4.02	8.10	10.67	-	-
9	1.41 ± 0.16 10 ⁷ ± 0.10	1.00 10 ⁷ ± 0.10	4.20	4.02	8.64	10.87	52.99	71.54

Starter culture *L. plantarum* B1765 showed:

- Increasing the growth of LAB to 10⁷ CFU/g higher than the control sample that was reached to 10⁶ CFU/g.
- Decreasing of pH to 4.02, whereas the control reached to 4.20.
- Increasing the peptide to 10.87 mg/g whereas the control reached to 8.64 mg/g.
- Increasing the ACEI activity to 71.54%, higher than control that was found to 52.99%.
- The culture starter *L. plantarum* B1765 could reduce the fermentation time 5 days shorter than control to get the same quality of bekasam product.

Conclusion:

- All the proteolytic LAB of bekasam isolate was found to result ACEI with the highest activity was given by *L. plantarum* B1765 and the lowest one was the *P. pentosaceus* B1661.
- The use of *L. plantarum* B1765 could improve the quality product of bekasam, reduced the fermentation time and increasing the ACEI activity.

Further Research Questions:

Further Research Questions:

- The ACEI activity was tested limited in vitro test so the study of in vivo is needed to evaluate the stability of ACEI substance to digestive enzymes.
- Isolation, purification and sequencing of bioactive peptides as ACEI is needed to develop this peptides as pharmaceutical or food supplement.

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Preparation of Bekasam :



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