The potential of lactic acid bacteria to improve the quality and number of carnocine during fermentation process of Bakasang as a functional food

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Abstract. The objectives of the research are to study the potensial of lactic acid bacteria to inhibit the growth histamine-producing bacteria, inhibit the formation of indicator component off-flavor of bakasang and increasing the amount of carnosine as a bioactive peptide. The results indicated that *Pediococcus* sp.B5.1 and *Pediococcus* sp.B1.0 were able to increase the amount of carnosine by 9.16 x and 8.92 x, able to inhibit the growth histamine-producing bacteria, soluble proteins increased by 10.4% and 10.5%, capable of inhibit the formation of histamine 5.8 mg / 100g and 5.9 mg / 100 mg, TVN (47.2 mg / 100gr and 45.6 mg / 100g), and TMA (24.2 mg / 100g and 22.6 mg / 100 g). This shows that the two isolates of BAL are able to improve the quality and maintain the safety of fermented foodsbakasang and that most the impotantly can increase the potential of bakasang to become functional food because it can increase the number of carnosine.

1. Introduction

Functional food is a food that able to have a beneficial effect on human health in addition to the effects of nutrition which in principle is owned by food. The term functional food was first introduced in the 80s and Japan decade, then in 1996 the Ministry of Health and Welfare of Japan (Ministry of Health and Welfare) issued a regulation on functional food known as FOSHU (Food for Specified Health Use) is food that has nutritional content certain for health purposes [6]. Functional foods are often referred to as foods that have health functions, especially for prevention of disease. Traditional fermented products with fish-based ingredients will become functional foods if there are substances that can have health effects such as carnosine.

Bakasang is a traditional fish fermentation product made from offal and eggs of skipjack fish (*Katsuwonus pelamis* L.) which added with salt [17];[9];[10]; [11], Tongkol Fish (*Euthynnus affinis*) [8]. Based on the raw material used, microbes involved and environmental conditions formed during the fermentation process, *Bakasang* is a fermented fish product that has the potential as a source for obtaining various types of lactic acid bacteria.

Lactic acid bacteria grow on protein-rich substrates; therefore, these bacteria have the ability to degrade and use protein as a source of nitrogen. Lactic acid produced from carbohydrate degradation will reduce pH which is useful in suppressing the growth of decomposing microbes and pathogens so that it can extend the shelf life of fermented products. The acidity of the product can also change the

texture due to the precipitation of several proteins and other biochemical changes that produce flavors that are typical of fermented products.

The potential use of fermented food for the development of functional foods is very promising especially high protein foods such as fish. Functional foods that are being developed at this time are foods that contain bioactive peptides that have the body's functional ability to maintain physiological performance and minimize the risk of degenerative diseases such as carnosine [3]. Carnosine is a dipeptide which composed of two amino acids, namely β-alanine-L-histidine which is abundant in fish protein. In this research it was suspected that lactic acid bacteria play a role in increasing the amount of carnosine. Carnosine has many benefits for the body's metabolic system [5] including functioning as an antioxidant and having the ability as a buffer capacity that can maintain the acidity of the intracellular environment due to muscle activity that produces lactic acid. Carnosine can also inhibit lipid oxidation and is able to protect cell membranes. The structure of Carnosine is thought to be relatively safe during the processing of food products including fermentation and heating because this peptide is relatively stable because it is protected by vellum on its surface so that it makes peptides (carnosine) free from acids and bases [18].

Therefore, the objectives of the research are to study the potensial of lactic acid bacteria to inhibit the growth histamine-producing bacteria, inhibit the formation of indicator component off-flavor of bakasang and increacingthe amount of carnosine as a bioactive peptide.

2. Materials and Methods

2.1. Starter Culture Lactic Acid Bacteria Isolate

Preparation of starter culture as much as 100 ml lactic acid bacteria. One ose of lactic acid bacteria was put into 5 ml of liquid MRS and incubated for 24 hours at 37^0C. Then, 5 ml of MRS which had been overgrown with lactic acid bacteria isolates were put into 95 ml of liquid MRS and re-incubated for 24 hours at 37^0C. 100 ml of lactic acid bacteria isolate containing 10^9cfu / ml is ready to be used as a starter.

2.2. Preparation of controlled fish fermentation (Bakasang)

Bakasang is made from Cakalang Fish which is separated between fish meat,stomach, and fish egg, then cleaned, drained and cut into small pieces. Before fermentation, all ingredients are added with 15% salt (b/b). The last stage was put into a bottle and incubated at 37^0C for 3 days.

2.3. Microbiolgical Analysis

Microbiological changes were carried out at the initial and final stages of fermentation. Micrbiological tests at each stage were carried out using dilussiondan plating methods by using selective media for the growth of certain bacteria. Media PCA is used to caunt total bacteria, media MRS is used to count lactic acid bacteria, media modified niven is used to count histamine-producing bacteria.

2.4. Chemical Analysis

The parameters measured (early and final) were Total of dissolved protein using Lowry Method [12], pH, Total of acid using AOAC method [1], number of histamines formed using Mahendrata method [13], TVN and TMA (indicator component off-flavor) using Conway diffusion method [2] and number of carnosine formed using Parker Method [15]

3. Result and Disscusion

3.1. The effect of pure culture (starter) of LAB isolate on microbiological changes

The controlled bakasangfermetation by using isolates of selected lactic acid bacteria namely Pediococcus sp.B5.1 and Pediococcus sp.B1.0 because these two isolates of LAB have the great

ability to affect changes in the number of microbes during fish fermentation (bakasang) and these two isolates also have a greater ability to increase the amount of carnosine. Data on changes in microbiology during bakasang fermentation process using 2 selected LAB isolates, Pediococcus sp. B5.1 and Pediococcus sp. B1.0, are presented in Table 1.

Based on data from Table 1, showed that the isolates of LAB Pediococcus sp. B5.1 and Pediococcus sp. B1.0 had a great ability to inhibit the growth of coliform bacteria and histamine-producing bacteria compared to controls. This is caused by lactic acid and metabolite products such as organic acids other than lactic acid, diasetil, hydrogen peroxide and bacteriocin produced by lactic acid bacteria as antimicrobial compounds that are inhibitory and even kill the growth of other bacteria that live in bakasang. This is also indicated by the increase in the number of lactic acid bacteria during the fermentation process of bakasang.

Table 1. Mikrobiological Changes during the Fermentation process using 2 (two) selected isolates of LAB

Isolate Code	Mikrobiological measurement CFUgr							
	Total Bacteria		Number of LAB		*Coliform*		Histamine-producing bacteria	
	early	final	early	final	early	final	early	final
Pediococcus sp. B5.1	$3.6x\ 10^6$	$8.2x10^9$	$4.6x10^6$	$8.3x10^9$	$1.5x10^5$	$2.5x10^1$	$3.1x10^5$	$2.8x10^2$
Pediococcus sp. B1.0	$3.9x10^6$	$8.1x10^9$	$4.9x10^6$	$9.2x10^9$	$1.7x10^5$	$2.3x10^1$	$3.2x10^5$	$2.3x10^2$
Control	$3.2x10^5$	$8.1x10^6$	$2.6x10^4$	$4.1x10^5$	$2.3x10^5$	$6.7x10^2$	$3.1x10^5$	$3.4x10^3$

Control : without LAB culture inoculation

Antimicrobial compounds cause changes in the permeability of the cytoplasmic membrane so that the membrane transport is disrupted, important enzymes become inactive and inhibit protein synthesis [4]. Organic acids will diffuse into bacterial cells in undissociated form. After entering the cell, the acid will dissociate because the cytoplasm has a high pH than outside the cell. Bacteria will regulate the internal pH to be neutral. To prevent conformational changes in the structure of cell proteins, enzymes and nucleic acids, protons that form in the cytoplasm from the results of acid dissociation must be released out of the cell. To remove protons from inside the cell, bacteria need high energy in the form of ATP. This proton removal process causes bacterial cell death due to running out of energy [4];[14]. This shows that the BAL isolates that play a role in the bakasang fermentation process are able to inhibit the growth of pathogenic bacteria and spoilage bacteria / bioaminant producers tested.

3.2. The effect of pure culture (starter) of LAB isolate on Chemical changes

LAB Isolate Pediococcus sp. B5.1 and Pediococcus sp. B1.0 which are inoculated have a large influence on chemical changes during the fermentation process of bakasang. Data on chemical changes that occur during the fermentation process are presented in Table 2 and Table 3.

Table 2. Chemical Changes during the fermentation process using 2 (two) selected isolates of LAB

Isolate Code	Chemical Changes					
	Total Acid (%)		pH		Dissolved Protein (%)	
	Early	Final	Early	Final	Early	Final
Pediococcus sp.B5.1	0.62	0.93	6.43	4.50	1.9	10.4
Pediococcus sp.B1.0	0.54	0.94	6.49	4.43	1.8	10.5
Control	0.62	0.82	6.53	5.63	1.8	8.6

Control : without LAB culture inoculation

Based on the data in Table 2a, both of LAB isolates namely Pediococcus sp. B5.1 and Pediococcus sp. B1.0 showed lower pH values of 4.5 and 4.43 compared to pH control values (5.63). The total titrated acid (%) of the two LAB isolates was higher at 0.93% and 0.94% compared to controls (0.82%). This proves that Pediococcus sp. of Lactic acid bacteria isolates. B5.1 and Pediococcus sp. B1.0 are homofermentative bacteria which in their metabolism use the glycolysis pathway (Embden-Meyerhoff-Parnas, EMP and produce lactic acid approximately 85% of glucose. Thus, both isolates of selected lactic acid bacteria have the ability to produce Lactic acid which can inhibit growth even kills histamine-producing bacteria, coliform bacteria and number of bacteria that do not damage food products. In Table 2a also shows that the two isolates of lactic acid bacteria have proteolytic ability which can be seen from changes in dissolved protein (%), namely LAB Isolate Pediococcus sp. B5.1 (from 1.9% to 10.4% and Pediococcus sp. B1.0 (1.8% to 10.5%). Changes in the dissolved protein (%) of the two LAB isolates were higher than the controls. This has a relationship with the increase in the amount of carnosine possessed by the two BAL isolates. In Table 2b, shows that the two isolates of LAB can inhibit the formation of histamine compared to controls. The off-flavor component indicators are TVN values (45.6-47.2 mg / 100g) and TMA (22.6-24.2 mg / 100g) were lower if compared with controls 89.6 mg / 100g (TVN) and 51.1 mg / 100g (TMA). This is related to the ability of lactic acid bacteria to inhibit the growth of histamine-producing bacteria.

Table 3. Chemical Changes during the fermentation process using 2 (two) selected isolates of LAB

Isolate Code	Chemical Changes					
	Histamin (mg/100g)		TVN (mg/100g)		TMA (mg/100g)	
	Early	Final	Early	Final	Early	Final
Pediococcus sp.B5.1	2,8	5,8	7,9	47,2	3,7	24,2
Pediococcus sp.B1.0	2,8	5,9	8,3	45,6	4,3	22,6
Control	2,6	10,5	6,8	89,6	4,2	51,1

Control : without LAB culture inoculation

3.3. The effect of pure culture (starter) of LAB isolate on increasing number of Carnosine during Bakasang fermentation

Both of LAB Isolates Pediococcus sp. B5.1 and Pediococcus sp. B1.0 can increase the number of carnosine compared to controls. This can be seen in Table 4.

Table 4. Enhancement number of Carnosine during fermentation process of bakasang using two selected isolates of LAB

Isolate Code	Carnosine (mg/100gr)		
	Early	Final	Enhancement
Pediococcus sp.B5.1	12.5	114.5	9,16 x
Pediococcus sp.B1.0	11.94	106.5	8,92 x
Control	12.7	13.1	Constant

Control : without LAB culture inoculation

The increase in the number of carnosine was formed as much as 9,16x and 8,92x during the bakasang fermentation process using Pediococcus sp. B5.1 and Pediococcus sp. B1.0 as stater indicate that this bakasang fermented food has the potential to be a functional food.

Enhancement in the number of carnosine during the fermentation process occurs also in research conducted by [7] and [16]; which showed an increase in carnosine in fermented Oyster (Crassostreagigas) Sauce as much as 18.7% and 28 µg / 500 L in the muscle of pigs.

Thus, BAL Isolate Pediococcus sp. B5.1 and Pediococcus sp. B1.0 can be used as a starter in bakasang fermented food because it has the ability to improve quality, product safety and especially can increase the amount of carnosine as much as 9.16 x and 8.92 x so that it has the potential to make

fermented food as functional food. This quality improvement is related to the ability of BAL isolates to inhibit spoilage bacteria and minimize the formation of off-flavor components, namely TVN and TMA.

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