Microbiological and chemical quality of a traditional salted-fermented fish (peda) product of Banten, Indonesia using *Leuconostoc mesenteroides* ssp. *Cremonis* BN12 as starter culture

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Abstract. Peda is a traditional salted fermented fish product of natural fermentation of salted mackerel fish of Indonesia. This study was carried out to evaluate the effect of *Leuconostoc mesenteroides* ssp. *Cremonis* BN12 as starter culture on the microbiological and chemical quality of peda. Peda was processed by fermentation either with or without *Leuconostoc mesenteroides* ssp. *Cremonis* BN12 2.5; 5.0 and 7.5 percent (v/w) with addition of 30% (w/w) salt at room temperature for 9 days. The observation was consist of microbiological analysis {total plate count, total lactic acid bacteria (LAB) and total Coliform} and chemical analysis (pH and total volatile bases-N/TVB-N). The result showed that *Leuconostoc mesenteroides* ssp. *Cremonis* BN12 could produce a better quality of peda than control, especially on starter concentration 5%. Total bacterial count 5.1 log CFU/g, total LAB count 4.0 log CFU/g, and Coliform 4 log CFU/g, respectively. Moreover, the TVB-N and pH value were 45.12 mgN/100g and 5.79, respectively.

1. Introduction

Indonesian fermented fish products are mostly produced according to local tradition and preferences. Therefore, substantial differences exist in the production methods, proportion and type of raw materials used. Peda is one of the fermented fish products made of fish prepared with the addition of salt. One of the functions of salt is to select microorganisms which produce proteolytic enzymes [1]. Peda is one of the semi-dried fermentation product without a further drying process during the production which allows the fermentation process continues. Generally, the process of peda fermentation is spontaneous fermentation, where microbes are not intentionally added as the starter culture, but they are an indigenous microbes from the environmental or raw material which spontaneously play an active role during the fermentation process [2,3]. Spontaneous fermentation of fish product generally utilize a high concentrations of salt to select certain microbes and inhibit the growth of microbes that cause product deterioration. Therefore only halophilic microbes will
survive [3].

*Leuconostoc mesenteroides* BN12 is a lactic acid bacteria isolated from *rusip* Bangka. The LAB type *Leuconostoc mesenteroides* is expected to accelerate the process of reducing pH during the anaerobic fermentation. Higher pH may allow microbes (including spoilage bacteria) grow well in the product. Most of microorganisms grow well at a pH around 5-8 and only certain types can survive at low pH [4]. Several studies on the spontaneous fermentation of peda have been reported [1,3,5]. The addition of a culture starter to the product during peda fermentation produces a good quality of peda [6].

However, the fermentation of peda utilizing a *Leuconostoc mesenteroides* bacteria as a starter culture has never been done by other studies. Therefore, in this study fermentation of peda using *Leuconostoc mesenteroides* as a starter culture was evaluated.

2. Materials and methods

2.1. Starter preparation

Pure culture of *Leuconostoc mesenteroides* ssp. *Cremonis* BN12 (LM BN12) isolated from *rusip* (a fermented-fish product from Bangka) was obtained from the stock culture of Research Center for Marine and Fisheries Product Processing and Biotechnology, Jakarta. One milliliter of LM BN12 culture transferred into 4 ml of CM lactose broth and was incubated at 35°C for 12 h. Five ml of the culture was then transferred into 95 ml of CM lactose broth and was incubated for 24 h. Finally, a hundred ml of CM lactose culture uses as a starter with the final concentration of 7 log CFU/ml. The experimental concentration of lactic acid bacteria were 0%, 2.5%, 5% and 7.5%. This procedure was repeated three times.

2.2. Preparation of salted fermented fish (peda)

The whole, fresh fish is cleaned, gutted and covered with salt and arranged in the layer by layer in the plastic boxes. The top layer is thicker than the lower layer of the salt. Further, it left to ferment during nine days anaerobically on the ambient temperature (28°C). Fermentation process showed in figure1.

2.3. Microbiological analysis

10 g of sample was homogenized with 90 ml of butter field phosphate (BFP) water for 30 s in a homogenizer and growth in Plate Count Agar (Oxoid) for Total Plate Count (TPC), deMan, Rogosa and Sharpe (MRS) agar plates (Oxoid) for total lactic acid bacteria, and Violet Red Bile Agar (VRBA) plates (Oxoid) for total coliform. Plates were incubated at 35°C. PCA, MRS, and H2S plates were incubated for 48 hours and VRBA for max. 18 hours. TPC, lactic acid bacteria count, and total coliform were measured on days 0, 3, 6, and 9 using SNI method [7].

2.4. Chemical analysis

pH and total volatile bases (TVB) were measured on days 0, 3, 6, and 9 using AOAC method [10].

2.5. Statistical analysis

This research used a completely randomized design with two factors and three replicates. Data were analyzed statistically using Analysis of Variance (ANOVA). Comparison of means was carried out by Duncan’s multiple range tests [9]. Statistical analysis was performed using SPSS statistic program (Version 11.0) for Window.
Figure 1. Flow chart of peda processing

3. Results and discussions

3.1 Microbiological changes

3.1.1 Effect of LMBN12 addition on Total Plate Count (TPC)
Analysis of total plate count (TPC) is one of determination test for microbiology quantity in a food product by counting a total number of microorganisms both bacteria, mold and yeast present in foodstuff [10]. The change of TPC during the fermentation could be seen in figure 2.
On the third day, fer on of peda with 30 lt showed the TPC was increased in all starter concentrations. TPC increased suspected due to growth of halophilic/halotolerant bacteria at the beginning of fermentation process. This study is in agreement with those obtained by [11], who reported that total plate count on rusip product increased on 3rd day. During the process of fermentation (salting) happens succession (turnover) dominance of bacteria in fish that was from Gram-negative to positive, such as lactic acid bacteria [12]. During the fermentation process, the most growing bacteria was lactic acid bacteria and remaining other halophilic bacteria that was resistant to high salt levels. Microorganisms that play a role in fermentation are lactic acid-forming bacteria, propionic acid-forming bacteria, acetic acid-forming bacteria, several types of yeasts and molds [13].

On the sixth day, the only starter with a concentration 5% leads to decrease of the total bacterial count. The addition of L. mesenteroides starter causes decreasing of total aerobic bacteria. Unclear antecedent is due to the presence of bacteriocin (dextran) from lactic acid bacteria. Otherwise, other treatments caused a stationery phase, which is the microbial growth is equal to the rate of microbial mortality. On the log phase, the nutrients that exist in the medium has been used maximally, with the result that the nutrition was needed for life on the stationer phase was wear off [14].

Fermentation pattern of peda using a starter is slightly different from without starter, especially after the sixth day. On fermentation without starter TPC values rise to day nine which is suspected due to the presence of other microbes that grow and multiply. Similarly with [3] generally, there is a decline in TPC log value from 0 days to 6 days fermentation, but increased after six days until 14 days of fermentation. Microbial growth occurs because of the energy, thus allowing microbes to form cellular components [14]. The formation of energy can be met if there is nutrients from the environment around the microbial cells are located or from food if the microbes live in food. On this condition, The best TPC value is treatment with 5% starter concentration.

3.1.2. Effect of LMBN12 addition on lactic acid bacteria (LAB)

In this research, there are also found lactic acid bacteria (LAB) as the dominant microorganism. This result is similar with [15] reported that lactic acid bacteria (LAB) are also seen as the predominant microorganisms in much fermented fish. A large number of microorganism are associated with the fermentation of peda [16]. Microflora of peda consisted of a variety of Gram-positive bacteria, i.e lactobacillus genera, leuconostoc, and Staphylococcus. Lanhouin, a peda-like fermented fish product, origin from the Republic of Benin, Africa predominant microorganisms were micrococci followed by bacilli like Staphylococcus aureus and Clostridium spp.[17].

Many authors have reported an extensive of microorganisms involved in fish fermentation in African regions. A large number of microorganisms are associated to the fermentation of lanhouin. The microbial population of lanhouin consisted of a variety of Gram-positive and Gram-negative bacteria [18].

The primary role of the LAB is to ferment the available carbohydrates and thereby cause a decrease in pH. The combination of low pH and organic acids (mainly lactic acid) is the main preservation factor.
in fermented fish products[15]. Change in LAB counts in peda inoculated with LM BN12 were shown in figure 3.

The LAB count with starter has a similar trend with the LAB count without a starter at day 3. The LAB count increased significantly, but the total LAB count with starter 1-1.5 log cycle was higher on the same day. The highest LAB count with starter occurred at day 3, but without starter occurred at day 9. These result showed that generally, LM BN12 addition effective to increased the LAB counts in peda. This starter dominated the microorganism population that was a role in peda fermentation. Fermentation of bekasam with Lactobacillus plantarum B1765 addition has the highest LAB count at day 3 [19].

During the fermentation process, the LAB count of peda increased on day 3, and then decreased at day 6, and that raised again at day 9. Similar to this research, contaminat microorganism by LAB significantly inhibited in olive fermentation, mackerel, corn silage [20].

3.1.3. Effect of LM BN12 addition on coliform
Coliform was indicator of bacteria for sanitation and hygiene of food. The source of coliform come from raw materials or the environment during processing or storage. Enteric bacteria were dominant bacteria in scombroid fish [21]. The change of coliform count in peda shown in figure 4.
The increasing of coliform bacteria in all treatment on the third day due to environmental condition of peda was suitable for them. The coliform count without a starter (day 9) was 5.6 log CFU/g, whereas the coliform count with the starter on the same day with 5% BAL was 4.84 log CFU/g. Besides, the coliform count with a starter of 2.5% and 7.5% showed decreasing of coliform count earlier (day 6) and increased again at day 9. Lowering of the coliform count was inversely proportional with LAB count. On the LAB concentrations 2.5% and 7.5%, the LAB count increased, so that coliform count decreased. Lowering of the coliform probably caused the lactic acid bacteria could suppress of coliform. The same results were shown that on the coliform count increased at the first week of fermentation peda [6].

Coliform was closely related to pH value, pH value of 0%, 2.5% and 7.5% LAB concentration increased at day 9. Meanwhile, the coliform count was directly proportional to the pH value. pH value of peda with 0%, 2.5% and 7.5% of LAB relatively higher than 5% of LAB. It was assumed pH value of the three treatments was suitable for coliform. pH value of these treatments were 5.8-6.0, while the optimum pH for growth of the coliform was 7.0 [22]. At 5% LAB, coliform count was 4.84. It showed that the addition of starter as much as 5% can reduce coliform.

3.2 Chemical changes

3.2.1. Effect of LM BN12 addition on pH change

pH value is a concentration of the hydrogen ions present in the solution which indicates the degree of acidity of a material. The pH value dramatically affects microorganisms that can grow on food so as it is crucial in determining the shelf life of food [10].

Changes in pH value during the fermentation process with the addition of L. mesenteroides starter can be seen in figure 5. Generally, pH value at peda decreased during 9 days fermentation. However, only 5% starter could decrease pH value at day 9. The pH value was inversely proportional to the total lactic acid bacteria. Suggested that the decrease in pH was caused by a reduction in the number of aerobic bacteria, which resulted in decreased ammonia release followed by the use of amino acids by fermenting microorganisms [18].
The addition of a starter causes a decrease in the pH caused by the production of organic acids (Figure 5). Starter cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid and also acetic acid [23]. In this research, there is a relation between the total lactate acid bacteria and pH value. Analysis result, on the ninth starter inoculation 5% could increase total LAB.

The pH values 5.86 formed in the fermentation process are in the range of 5.80-5.98. The pH value is suspected bacteria found in the peda product is a group of lactic acid bacteria. Reported that lactic acid bacteria grow in a low pH [24]. Therefore, food that has a lower pH will be more durable as fewer types of microorganisms can grow. The minimum pH value for the growth of microorganisms is affected by the kind of acid present in the food. This study showed that the addition of L. mesenteroides desacidic acid lactate starter could accelerate the occurrence of pH decrease, so the length of fermentation can also be accelerated. The statistical test results stated that the concentration of BAL is not significantly different (> 0.05). Starter causes rapid acidification of the raw material through the production of organic acids, mainly lactic acid [23]. Fermentation of the lactic acid bacteria also produce acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes. The standard pH requirement for fermented fish as Pedah Siam in Thailand is 6.0-6.4 with a pH 6.5 or higher being considered as a poor quality [25]. Meanwhile, in Indonesia, there is no recommended pH range for the salted-fermented fish product as rupis yet by Indonesia Food and Drug Authority. The high pH values of salted fermented fish products could be attributed to the high level of total volatile basic nitrogen (TVB-N) [26].

3.2.2. Effect of LM BN12 addition on Total volatile base (TVB)

TVB was the index of deterioration for freshwater fish and fermentation product of fish [27]. TVB is a parameter used to see the freshness of fish and has significance in the process of deterioration of fish [3]. Analysis for TVB involves the estimation of all volatile amines produces during spoilage. TVB value would estimate the amount of ammonia (NH₃) present, which is formed mainly as a product of protein breakdown, the amount of dimethylamine, as well as the TMA present [28].

TVB value in all treatment with the starter was decreased, contrary to treatment without using a starter. Decreasing in TVB value may be occured by salt adding in process. Salt suppresses the growth of decay-causing microorganisms. Changes in TVB values during the fermentation process of the peda
with the addition of *L. mesenteroides* starter can be seen in figure 6. Based on this research, on the 3rd day fermentation has increased in all treatments.

![Figure 6. TVB value of peda during fermentation on 0% starter (control), 2.5% starter, 5.0% starter and 7.5% starter](image)

TVB values on the fermentation of the control pause continued to increase on the 9th day while the pauses using the starter decreased until the 9th day. The final result of TVB value was peda with starter 2.5% 47.81 mgN/100g, 5% equal to 45.12 mgN/100g and 7.5% equal to 35.76 mgN/100g. The result indicates that the addition of *L. mesenteroides* as a starter can suppress the rate of TVB formation. The same result was shown that fermented plaque the addition of *L. plantarum* B1765 starter did not show a significant difference of TVB count from day one of 42.93 mgN/100g until the seventh day of 44.80 mgN/100g [18]. Spontaneously fermented TVB values tended to increase significantly from 69.07 mgN/100g on the first day fermentation to 274.40 mgN/100g on the fourth day of fermentation. Furthermore, from the fourth day until the seventh day TVB levels did not show significant differences.

According to [29], TVB is an indicator of fish quality, TVB value 200 mg/100g is a feasible limit consumed. Compounds classified as trimethylamine, dimethylamine, ammonia and other nitrogenous bases are the work of bacteria and autolytic enzymes during the process of decomposition. Increasing TVB content is related to the number of bacteria that can break down proteins into simple nitrogen compounds and volatile bases [30]. Salt may inhibit the growth of decaying microorganisms but it may not with a high salt content. The result of statistical analysis showed no difference (P>0.05).

4. Conclusion

*Leuconostoc mesenteroides* ssp. *Cremonis* BN12 could produce a better quality of peda, especially on starter concentration 5%. Total bacterial count 1.19x10^8 CFU/g, total LAB count 1.01x10^4 CFU/g, and *coli*form 7x10^3 CFU/g. Moreover, TVB was 45.12 mgN/100g and pH value was 5.79.

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