Chemical and microbiological characteristics of Pacik Kule, a traditional fermented carp from Southeast Aceh

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Abstract. Pacik kule is a dish made from carp (Cyprinus carpio) which is commonly found in Southeast Aceh as traditional food. Before being processed into pacik kule, carps are fermented for 1 to 2 days at room temperature using salt or without salt. This study aims to analyze the effect of salt addition and fermentation time on the chemical and microbial characteristics of pacik kule. This research used factorial completely randomized design consisting of 2 factors. The first factor was the fermentation time which consists of 2 levels, namely 1 day (K1) fermentation, and 2 days (K2) fermentation. The second factor is the addition of salt consisting of 2 levels, which is S1 (without salt) and (S2) with salt (1.5%). The analysis conducted is a chemical analysis and microbiological analysis. The results showed that the length of fermentation affected the total lactic acid bacteria but did not affect other parameters such as protein content, total volatile bases, and pH. The addition of salt affected the water content and total lactic acid bacteria but did not affect protein content, pH, and total volatile bases (TVB). The interaction between length of fermentation and salt addition affected water content, protein content, total volatile bases (TVB), and total lactic acid bacteria. Based on the results of chemical and microbiological analysis, fermentation for 1 day with the addition of salt (1.5%) was the best treatment with the highest protein content (11.93%), total lactic acid bacteria (9.4 log CFU/g), water content the lowest (17.41%) and the lowest total volatile base (13.63 mgN / 100g).

1. Introduction

Indonesia has a variety of cultures, traditions, and characteristics, especially the characteristics of traditional foods that are diverse from various regions. One of these traditional food is processed fermented fish. Fish is an animal that is consumed by many people and available at an affordable price. Fermented fish products in Indonesia have different forms, raw materials, and types of fermentation and generally still use a spontaneous process that is fermentation using salt [1, 2]. According to [3], fermented food products usually contain higher nutritional value than the original ingredients. This result can be caused by microbes in fermentation products that can break down complex components in food into simpler ingredients, easier to digest and also microbes can synthesize some vitamins.

Fish fermentation products that are well-known in Indonesia include Picungan, a traditional fermented fish product that is processed using picungan seeds (Pangium edule reinw) originating from Banten [4], Terasi which is a traditional fish fermentation product found in Cirebon [5], Pakasam originating from South Kalimantan, made from fish marinated through salt fermentation process [6], and Wadi which is a fish fermentation product originating from Central Kalimantan [7]. In Aceh, fish fermentation products that have been studied are bude and belacan depik. Bude is one of the fermented fish products which is
processed using anchovy (Stolephorus sp) and salt as raw materials. It has a rather thick liquid form, that can be consumed as side dishes in a raw or processed condition [1]. *Belacan depik* is one of the fermented fish products in the form of pasta, which is made from several raw materials including *depik fish* (*Rasboraawarensis*), laos, lemongrass, turmeric, and salt, which can be consumed as seasonings in the processed form [8].

One of the other fermented fish products that have not been studied is *pacik kule*. Based on the results of a field survey (2019), *pacik kule* is a dish made from carp (*Cyprinus carpio*) which is widely found in Southeast Aceh as traditional food. Before being processed into *pacik kule*, caps are fermented for 1 and 2 days at room temperature using salt or without salt. The variation of salt used in fermentation is around 1% to 2%. *Pacik kule* is taken from a combination of Alas and Gaya language where *pacik* means rotten and *kule* means tiger. *Pacik kule* means processed fish that are not good or rotten and then reused to becomes a traditional food that can be consumed. The word *pacik kule* comes from the story of a tiger that has a habit of spoiling food before eating. That is the beginning of the story of the process of *pacik kule* name.

Some changes occur during the fermentation process such as in the aroma, color, and texture. These changes were indicated by the smell of acid, the color begins to turn pale gray, and the texture begins to soften. According to [9], the principle of preservation in fish fermentation products is caused by several factors consisting of a decrease in water activity by salt and a decrease in pH due to the formation of acid by microbes. Fermentation aims to select spoilage and pathogenic microbes with the result that the microbes contained in fish during fermentation are microbes that are resistant to salt conditions and can play a role during fermentation.

The presence of salt can affect the types of microorganisms that play a role during the fermentation process. Different salt concentrations can affect microbial growth and safety of fermented products. Optimum growth of lactic acid bacteria is depending on salt concentrations that are not higher than 6% to 7% for *ppla-som* fermentation that occurs for 4-7 days [10]. *Plaa-som* is a fermented fish product that is generally made by Thai people, which is traditionally made using raw materials from freshwater fish or sea fish. Salt fermentation can also cause changes in the chemical and microbiological of fish meat. Therefore, this study aims to determine the changes in chemical and microbiological of *pacik kule* during fermentation with the addition of salt.

## 2. Materials and Method

### 2.1 Materials

The main raw materials used in this study are carp and salt. *Pacik kule* was made based on procedures carried out by the people of Southeast Aceh. The chemicals used in the analysis were aquades, de Man Rogosa Sharpe Agar (MRSA), natrium agar (NA), Pepton, NaOH, HCl, HgO, K2SO4, H2SO4, HBO3, H3BO3, NaOH-Na2S2O3, K2CO3, TCA, methyl red, methylene blue, phenopthalein indicator, and alcohol. The tools used in the process of making *pacik kule* are plastic jars, knives, and digital scales. The tools used to analyze were petri dishes, pipette drops, spatulas, incubators, test tubes, measuring cups, erlenmeyers, analytical scales, ovens, desiccators, burettes, destruction, kieltec system, pH meters, colony counters, filter paper, titration, furnaces, blender, outer chamber, digital refractometer, measuring flask, kjeldhal flask, condenser, bunsen, and laminar flow cabinet.

### 2.2 Experimental Design

This study used a completely randomized design with two independent variables. The first variable was the length time of fermentation being used (K1= 1 day and K2 = 2 days) whilst the second variable was the salt addition (S1=without salt and S2= addition 1.5% salt. Then all the treatment combinations were done in three repetitions, resulting the total experiment units of 12 samples. Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used for statistical analysis.
2.3 Research Procedure
The process of fermented pacik kule was performed with common procedures carried out by the people of Southeast Aceh, with variations in salt concentration and different length of fermentation. The first step of the procedure was removing the gills, bones, head, and fins of carp. Furthermore, the fish is washed, then drained, weighed and added with salt according to the treatment (without salt and salt addition (1.5%)). Finally, at around 1500 grams of fish meat is put in a tightly closed jar and fermented at room temperature according to the treatment (1 day and 2 days). The analysis conducted in this study was the water content [11], protein content with Kjedahl method [11], pH [12], total volatile bases (TVB), and total lactic acid bacteria [13].

3. Result and discussion
3.1 Moisture Content
The value of moisture content ranged from 17.41% to 23.25% with an average of around 20.80%. Analysis of variance shows that the interaction length of fermentation and the addition of salt have a significant effect (P≤0.05) on the moisture content of pacik kule (Figure 1). Figure 1 shows the comparison between treatments without salt has a higher water content value (23.25%) compared to the treatment of adding salt (1.5%) around 17.41%. The function of salt in the fermentation process aims to reduce the water content in the material so that the moisture content in food will be reduced and can inhibit bacterial growth. According to [14], a decrease in water content occurs due to the salting process carried out in the fermentation process, because salt can attract water molecules that exist in fish tissue.

![Figure 1](image.png)

**Figure 1.** Interaction of length of fermentation and salt addition towards moisture content of pacik kule at DMRT$_{0.05}$ level 1 = 3.31, level 2 = 3.44, level 3 = 3.52, and coefficient of variants = 8.44% (Values followed by the same letter indicate no significant difference)

3.2 Protein content
Protein content ranged from 9.18% to 11.93% with an average of 10.38%. Based on the results of ANOVA, the interaction period of fermentation and addition of salt (KS) has a significant effect (P≤0.05). Based on Figure 2, it can be seen that the protein content of pacik kule in the first day of fermentation is around 11.25% compared to the second-day fermentation which is around 9.17%, it is assumed that the longer the fermentation time, the lower the value of the protein produced. According to [15] decreasing protein value during fermentation occurs due to protein hydrolysis to amino acids and peptides, and then amino acids will be further decomposed into other components that play a role in the formation of product flavors. The increase in the value of protein content in salt addition (1.5%) is around 11.93%, due to the low water content in this treatment. The previous study by [16] claims that water content is inversely
proportional to protein content. This result is in accordance with the research by [17], that the higher the water content produced from food, the lower the protein will be due to myogen and protein dissolved in water.

Figure 2. Interaction of length of fermentation and salt addition towards protein content of pacik kule at DMRT0.05 level 1 = 3.07, level 2 = 3.19, level 3 = 3.27, and coefficient of variants = 15.7% (Values followed by the same letter indicate no significant difference)

3.3 Total Volatile Bases (TVB)

TVB value is a parameter used to notice the freshness of fish and has an important meaning in the process of deterioration of fish quality. TVB values of pacik kule ranged from 13.63 to 32.68 mg N/100g with an average value of 23.77 mg N/100g. Analysis of variance shows the interaction length of fermentation and salt addition has a very significant effect (P≤0.01) on the value of TVB pacik kule. Figure 3 shows that the total volatile bases of pacik kule which is fermented for 2 days without salt are higher (32.68 mgN/100g) compared to salt addition fermentation (1.5%) for 2 days with a value of 24.65 mgN/100g. The low value of TVB in the addition of salt treatment (1.5), presumably due to the addition of salt inhibits decay during the fermentation process. The previous study by [18] explained that the rusip fermentation added with palm sugar has a lower TVB value. The presence of the lactic acid bacteria in rusip fermented can suppress the growth of TVB-forming microbes.

The high salt content can be used as a preservative and can inhibit the growth of microorganisms, such as decomposing microorganisms that will produce alkaline compounds (TVB). The longer fermentation time usually caused the TVB detected levels higher. This result can be caused by the accumulation of metabolites of microorganisms including TVB in food products that indicate the occurrence of decay [19].
Figure 3. Interaction of length of fermentation and salt addition towards total volatile bases of pacik kule at DMRT level 1 = 8.86, level 2 = 9.21, level 3 = 9.44, and coefficient of variants = 19.8% (Values followed by the same letter indicate no significant difference)

3.4 pH
The pH value is the main indicator that can determine the success of the fermentation process. The pH value of pacik kule during the fermentation process changed from initially 6.42 to 6.07. In this study, the results of variance for the pH of pacik kule showed that the influence of fermentation time (K), the addition of salt (S), and interaction time of fermentation and addition of salt had no significant effect (P≥0.05).

Previous research by [20] mentioned that the pH value of fresh fish ranged from 5.80 to 6.94, while during the fermentation process it ranged from 6.60 to 5.20 so that it can be seen that the pH obtained in this study was not much different from the pH from [20]. Furthermore, a study by [21] explained the longer the fermentation process, the pH value will decrease. At the time of the fermentation process, glucose will be converted to eventually become lactic acid, thereby increasing levels of lactic acid and causing pH values to decrease. Changes in pH that occur in the fish meat are caused by the process of autolysis and bacterial attack [22].

3.5 Total Lactic Acid Bacteria (LAB)
Lactic acid bacteria are a class of Gram-positive bacteria, which do not form spores in their life processes. These bacteria are common found in fermented foods to control fermentation. They contribute to the sensory and nutritional characteristics of fermented products [23-25]. Lactic acid bacteria can break down carbohydrates (sugar) and produce lactic acid. The results of the total value of lactic acid bacteria ranged from 5.54 log CFU/g to 9.10 log CFU/g, with an average value of 7.96 log CFU/g. Analysis of variance showed that the length of fermentation (K) and the addition of salt (S) had a very significant effect (P≤0.01) on the total lactic acid bacteria pacik kule.

Figure 4 shows the number of lactic acid bacteria on two days fermentation and without salt treatment had the lowest number of total lactic acid bacteria (5.54 log CFU/g) compared to other treatments. This result is suspected because lactic acid bacteria quickly adapt to the environmental conditions of growth on the first day, and the trend on the second day was a decline because nutrition has started to decrease. The previous study by [26] explained that the number of initial microbial cells will accelerate the adaptation phase and during the fermentation process, resulting in an increase in the total number of microbes. The high amount of lactic acid bacteria at the beginning of making rusip causes the breakdown of sugar into lactic acid is greater, and as the result, microbes that cannot stand with acid will be dying.
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Figure 4. Interaction of length of fermentation and salt addition towards total volatile bases of pacik kule at DMRT0.05 level 1 = 2.85, level 2 = 2.96, level 3 = 3.04 and coefficient of variants = 17.24% (Values followed by the same letter indicate no significant difference).

Furthermore, the increasing number of lactic acid bacteria to the treatment of adding salt can be caused by the presence of a salt solution that can stimulate the growth of lactic acid bacteria [27]. Research conducted by [28] explains that the higher the amount of glucose concentration used in the fermentation process, the higher the amount of lactic acid and halophilic bacteria produced. The use of salt and a tightly closed container in the process of making belacan depik affects the growth of lactic acid bacteria [8].

4. Conclusion
The longer the fermentation time, resulting in the lower value of the moisture content, protein content, and total lactic acid bacteria. The higher the salt concentration is used, the higher the protein content and total lactic acid bacteria. The best treatment was obtained from treatment with one-day fermentation and with the addition of salt (1.5%) due to the highest protein content of 11.93%, the highest total lactic acid bacteria 9.4 log CFU/g, the lowest amount of water content (17.41%) and the lowest total volatile base (13.63 mgN/100g).

5. References
[1] Muzaita, M. and N. Arpi 2015 Jurusan Teknologi Hasil Pertanian. Fakultas Pertanian
[2] Amri, M 2007 Jurnal Ilmu-Ilmu Pertanian Indonesia. 9, 71-76.
[3] Berlian, Z., Syarifah, Imamul, Huda 2016 Jurnal Biota. 2, 162-157.
[4] Heruwati, E. S, Widyasari, H. E., Haluan, J 2007 Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan. 2, 9-18.
[5] Romadhon, Rianingsih, L., Aanggo Apri Dwi 2018 Jurnal Pengolahan Hasil Perikanan Indonesia. 21, 68-76.
[6] Hestiyana 2017 Jurnal Totobuang, 5, 255-269.
[7] Rizky, M. Y., Fitri, R.D., Hastuti, U.S., Prabaningtyas, S 2017 Jurnal Bionature. 18, 87-98.
[8] Muzaita, M 2015 Jurnal Sagu 14, 19-22.
[9] Candra, J.I 2006 Skripsi. Fakultas Perikanan dan Ilmu Kelautan Institut Pertanian Bogor.
[10] Desniar, Poernomo., D, Wijayatur, W 2009 Jurnal Pengolahan Hasil Perikanan Indonesia. 12, 73-87.
[11] Association of Official Analytical Chemist (AOAC). 2015. Official Method of Analysis. 16th edition. Washington
[12] Association of Official Analytical Chemist (AOAC). 1995. *Official Method of Analysis.* 18th edition, Washington

[13] Fardiaz, S. 1989. *Mikrobiologi Pangan.* PAU Pangan dan Gizi. IPB.

[14] Mumitianah, O. N., Kusdiyantini, E., Budiharjo, A 2014 *Jurnal Biologi.* 3, 20-30.

[15] Adawayh, M.P. 2006. *Pengolahan dan Pengawetan Ikan.* PT. Bumi Askara, Jakarta.

[16] Suyatno, Sari. N.I., Loekman, S 2015 *Jurnal JOM.* 3,1-8.

[17] Hadiwiyoto, S. 1993. *Teknologi Pengolahan Hasil Perikanan Jilid 1.* Liberty, Yogyajakarta.

[18] Koeseoemawardani, D., dan Yuliana, N. 2009. *Jurnal Sains dan Teknologi Indonesia.* 11, 205-211.

[19] Dissaraphong S., S. Benjakul and W., Visessanguan 2006 *Bioresource Technol.* 10, 77-88.

[20] Desnair, Iriani, S., Retno, S.S 2002 *Jurnal Pengolahan Hasil Perikanan Indonesia.* 15, 232-239.

[21] Asma., Nisa, K., Wardani, A.K. 2016. *Jurnal Pangan dan Agroindustri.* 4, 367-376.

[22] Suptijah, P., Yayandi, Gushagia., Dadi Rochmadi Sukarsa 2008 Fakultas Perikanan dan Ilmu Kelautan. IPB : Bogor.

[23] Mayo B, Aleksandrak-Piekarczyk T, Fernandez M, Kowalczyk M, Alvarez-Martin P, Bardowski J, 2010. Updates in the Metabolism of Lactic Acid Bacteria, pp. 3-33. In Mozzi F, Raya RR and Vignolo G (Eds.) Biotechnology of Lactic Acid Bacteria Novel Application, Wiley-Blackwell, Iowa, USA.

[24] De Vuyst, L., G. Vrancken, F. Ravyts, T. Rimaux and S. Weekx 2009 *Food Microbiol.* 26, 666–675

[25] Felis GE, Dellaglio F 2007 *Microbiol.* 8, 44–61.

[26] Koeseoemawardani, D., Rian, S., Tauhid, M 2013 *Jurnal Aritech.* 33, 265-271.

[27] Anwar L. O., L Hardjito, dan Desnai 2014 *Jurnal pengolahan hasil perikanan Indonesia* 17, 254-262.

[28] Killinc, B., S. Cakli, S., Tolasa and T. Dincer 2006 *European Food Research Technology.* 222, 604-613.