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Total Lactic Acid Bacteria and pH Values in Lu'at Fermentation with Different Salt Levels and Fermentation Duration

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ABSTRACT

Cayenne pepper (*Capsicum frutescens* L.) is an important commodity in Indonesian cuisine for cooking spices and chili sauce. *Sambal* is in demand as a condiment with a spicy taste. *Lu'at* is a typical chili sauce from Timor, East Nusa Tenggara, which is used as a condiment with a spicy taste and is prepared by traditionally fermenting chilies, lime, salt, and other additional ingredients. The fermentation process involving lactic acid bacteria (LAB) can enhance the nutritional and sensory qualities of traditional fermented foods. However, limited research exists on the optimization of salt concentration and fermentation time for *lu'at* production. This research aims to determine the total number of lactic acid bacteria and the pH value of *lu'at* fermented with different salt levels and fermentation times. This study used a two-factorial Completely Randomized Design (CRD), namely salt content (G) (0%, 5%, and 10%) and fermentation time (F) (0, 7, and 14 days) with 3 repetitions. The data obtained were analyzed using Analysis of Variance (ANOVA), and if there were significant differences, it was continued with the Tukey Test. *Lu'at* was made from a mixture of crushed cayenne pepper, lime slices (*Citrus aurantifolia*), and basil leaves (*Ocimum basilicum*) in a ratio of 150:10:5. The results showed that there was a significant difference in the average total lactic acid bacteria between treatments with 5% and 10% salt content ($P<0.05$). However, there was no significant difference ($P>0.05$) in the treatment with fermentation time and the interaction between salt content and fermentation time. The highest total of lactic acid bacteria was in the treatment with a salt content of 5% and a fermentation time of 7 days (4.3×10^7 CFU/g). The pH value in *lu'at* fermentation was not significantly different in all treatments ($P>0.05$) with an average pH value range of 3.57–3.98. These findings suggest that 5% salt concentration with 7-day fermentation provides optimal conditions for LAB growth in *lu'at* production, contributing to the development of standardized traditional fermented condiment processing.

Keywords: *lu'at, fermentation, lactic acid bacteria (BAL) pH value, salt content*

INTRODUCTION

Cayenne pepper (*Capsicum frutescens* L.) is one of the important commodities in Indonesia because it has high economic value and selling price, even though it has a price that often fluctuates. Cayenne pepper has a spicy taste that can be used as a cooking spice (Wisnujati & Siswati, 2021). In addition to cooking spices, chili peppers are usually processed into chili sauce, chili jam (Rahmah & Ansori, 2023; Surya & Tedjakusuma, 2022), chili flour (Sebayang et al., 2019) , raw materials for making pickles (Novitasari, 2018), and so on. Chili sauce can be in the form of sauces, pastes, or seasonings made from chili peppers as the main raw material and other additional raw materials, served as condiments or side dishes (Rahmah & Ansori, 2023; Surya & Tedjakusuma, 2022). Indonesia has various types of chili sauce made from various types of chili peppers and other additives that vary according to local culture (Surya & Tedjakusuma, 2022). The process of making chili sauce in general includes washing, cleaning, and grinding, and can also be processed by cooking (Rahmah & Ansori, 2023). One type of chili sauce typical of Timor - East Nusa Tenggara (NTT) is called *lu'at*. This chili sauce has

become famous along with the increase in tourism and local culinary consumption in NTT, especially as a culinary accompaniment in NTT (Nugraha & Balukh, 2022; Setiawan & Giri, 2016; Bagaihing & Mantolas, 2021). *Lu'at* comes from the *Dawan* language (Timor Island), which means chili sauce. This chili sauce is processed from chili, lime, salt, and other raw materials with a traditional fermentation process Anggraeni et al., (2021) and is categorized as a cooked ingredient (Rahmah & Ansori, 2023). The organoleptic properties of *lu'at* include a slightly whitish red color, a slightly unpleasant aroma due to fermentation, a mushy texture, and a somewhat preferable taste (Naisali & Bria, 2022). In *lu'at* fermentation, the addition of salt and the length of the fermentation process can affect the color, aroma, taste, and texture of the *lu'at* (Naisali & Bria, 2022). Because the ingredients and processing of chili sauce are very diverse, chili processing as chili sauce has the potential to be developed to extend the shelf life of chili peppers and preserve Indonesian culinary specialties.

Lactic acid bacterial fermentation is a type of fermentation that has been widely carried out with simple and inexpensive techniques at the traditional household level. Lactic acid bacteria are heterotrophic microorganisms, so they need a nutrient-rich environment to live. Lactic acid bacteria ferment carbohydrates homofermentatively, which produces lactic acid, or heterofermentatively, which produces a mixture of carbon dioxide, lactic acid, acetic acid, and/or alcohol (Bagaihing & Mantolas, 2021). The results of testing on Salted Fermented Chili Pepper (SFCP) from China found lactic acid bacteria dominated by *Lactobacillus*, *Weissella*, *Pediococcus*, *Pseudomonas*, and *Enterobacter* bacteria (Liu et al., 2023). In the *Zha-chili* ferment, the dominant bacteria found were *Lactobacillus*, *Pseudomonas*, *Pediococcus*, *Weissella*, and *Staphylococcus* (Cai et al., 2021). Lactic acid bacteria and yeast play an important role in the fermentation of chili peppers in transforming pectin, lipids, proteins, carotenoids, capsaicinoids, and phenolics, resulting in distinctive aromas, amino acid flavors, organic acids, colors, textures, and components that are useful for health (Li et al., 2023).

Previous research on fermented chili products has primarily focused on Asian fermented chili preparations such as Chinese SFCP and *Zha-chili*, with limited attention to Indonesian traditional fermented chili products. Studies by Liu et al. (2023) provided comprehensive reviews of fermented chili pepper products globally but did not include detailed analysis of Indonesian traditional preparations like *lu'at*. Research by Cai et al. (2021) and Li et al. (2023) explored bacterial diversity and metabolic pathways in Chinese fermented chili products but focused on different raw materials, processing methods, and environmental conditions than those used in Indonesian traditional fermentation. While Anggraeni et al. (2021) examined salt concentration effects on vegetable fermentation, their work did not specifically address chili-based fermented products or the unique combination of ingredients found in *lu'at*.

The use of salt can also support the growth of lactic acid bacteria Wulan, (2023) and suppress the growth of pathogenic bacteria that can damage fermented products (Aristyan et al., 2014). The growth of lactic acid bacteria accelerates at low salt concentrations but is also inhibited at high concentrations, so optimal salt concentrations are needed to support the growth of lactic acid bacteria and suppress unwanted bacterial growth (Anggraeni et al., 2021). The growth of lactic acid bacteria that is inhibited in fermented foods with excessively high salt levels can also reduce the ability of lactic acid bacteria to produce lactic acid (Margiati et al., 2024). Salt is hygroscopic, so it can draw water from food and microbial cells, affect the moisture content of food through osmotic dehydration, select microbes that can grow on the

fermented food, and inhibit most proteolytic enzymes that are precursors to the growth of lactic acid bacteria, giving it taste and aroma (Yusra, 2023). Some traditional fermented foods use salt in varying amounts. For example, in the fermentation of fishery products, using high enough salt levels can suppress the growth of unwanted microbes, such as *chao* using salt of 20% w/w Matti et al., (2021), shrimp paste with a salt content of 20-25% of the total weight Mutamimah et al., (2023), and *budu* with a salt content of 30% of the weight of the fish (Anggraeni et al., 2021). Meanwhile, fermentation in vegetables uses lower salt levels ranging from 2-12.5% [20]. Salt in fermented foods needs to be considered because it affects the physicochemical and microbiological quality of the food (Anggraeni et al., 2021).

The research gap specifically lies in the absence of systematic studies examining the relationship between salt concentration and fermentation duration on the microbiological quality of *lu'at*. While numerous studies have investigated salt effects on various fermented foods, there is limited research on how these parameters interact to influence LAB growth and pH development in traditional Indonesian chili-based fermented products. This gap is particularly significant given the unique ingredient composition of *lu'at*, which includes lime and basil in addition to chili and salt, creating a distinct fermentation environment that may respond differently to salt concentration compared to other fermented products.

The novelty of this research lies in its systematic investigation of *lu'at* fermentation parameters, representing the first scientific study to quantify LAB populations and pH changes during *lu'at* fermentation under controlled conditions. Unlike previous studies that focused on established fermented chili products, this research explores a traditional Indonesian product with unique ingredient combinations and processing methods. The factorial experimental design examining both salt concentration and time effects provides comprehensive insights into optimal fermentation conditions for this culturally important food product.

So far, research on *lu'at* has been limited to organoleptic trait tests Naisali & Bria, (2022) and its role in supporting Timorese culinary tourism in East Nusa Tenggara (Nugraha & Balukh, 2022). Although several types of lactic acid bacteria have been found in other chili fermented products (Cai et al., 2021; Al Rahmad et al., 2023), there have been no microbiological studies related to total lactic acid bacteria in *lu'at* at different salt concentrations and fermentation times. Therefore, this study was conducted with the aim of determining the total lactic acid bacteria and pH values in fermented *lu'at* with different salt levels and fermentation duration.

The research problem addressed in this study centers on the limited scientific understanding of the fermentation processes in *lu'at*, a traditional food product with significant cultural and economic importance in East Nusa Tenggara. While *lu'at* has been traditionally produced for generations, there is insufficient knowledge about the microbiological mechanisms underlying its fermentation, particularly how salt concentration and fermentation duration affect the growth of beneficial lactic acid bacteria and the development of optimal pH conditions. This knowledge gap hinders the development of standardized production methods and quality control measures for *lu'at* manufacturing.

The urgency of this research stems from the growing interest in traditional fermented foods as functional foods with potential health benefits, combined with the need to preserve and scientifically validate traditional food processing methods. As *lu'at* gains recognition in the tourism and culinary sectors of East Nusa Tenggara, there is an immediate need for

evidence-based production guidelines that ensure consistent quality, safety, and nutritional value. Furthermore, understanding the optimal fermentation conditions is crucial for scaling up production to meet increasing market demand while maintaining the traditional characteristics and health benefits of this indigenous food product.

The specific objectives of this study are: (1) to quantify the total lactic acid bacteria populations in *lu'at* under different salt concentrations (0%, 5%, and 10%) and fermentation durations (0, 7, and 14 days); (2) to evaluate pH changes during *lu'at* fermentation across different treatment combinations; (3) to determine the optimal salt concentration and fermentation time for maximizing LAB growth; (4) to establish the relationship between LAB populations and pH values during fermentation; and (5) to provide scientific evidence for standardizing *lu'at* production processes.

The benefits of this research include providing scientific validation of traditional *lu'at* fermentation practices, establishing optimal processing parameters for consistent product quality, supporting the development of commercial production standards for *lu'at* manufacturing, contributing to the preservation of traditional Indonesian food knowledge through scientific documentation, and offering baseline data for future research on the nutritional and functional properties of *lu'at*. The implications extend to food manufacturers, traditional food processors, tourism industry stakeholders, and researchers interested in Indonesian traditional fermented foods.

METHOD

This study is an experimental study using a completely randomized design of 2-factorial with the first treatment of salt content (G) consisting of 3 levels (0%, 5%, and 10% w/w), and the second treatment of fermentation duration (F) consisting of 3 levels (0, 7, and 14 days). Each treatment was repeated 3 times so that 27 experimental units were obtained. This research was conducted at the Food Laboratory and Microbiology Laboratory, Faculty of Science and Technology, Widya Mandira Catholic University Kupang. The research period was during May-June 2024.

The ingredients used in this study were cayenne pepper (*Capsicum frutescens* L.), lime (*Citrus aurantifolia*), and basil leaves (*Ocimum basilicum*) obtained from the Oeba Traditional Market, table salt (Ship), 70% alcohol (Biomed), aquades, 0.1% Buffered Peptone Water (Oxoid), deMan-Rogosa-Sharpe Agar (MRS Agar) Media (Merck), tissue, cotton, *spiritus*, and aluminum foil. The tools used in this study consisted of chopper (Mitochiba CH 200), basin, cutting board, knife, glass jar, spoon, bowl, analytical scale (Otsuka), spatula, stirring rod, electric stove (Oxone), autoclave (Hirayama), spirit lamp, laminar air flow, inoculating needle, Drigalski spreader, petri dish, micropipette (Eppendorf), pipette tip (Eppendorf), sample plastic, Erlenmeyer flask, measuring cup, glass cup, test tube, tube cover, vortex (DLAB MX-S), pH meter (PHS-3C), and incubator (Memmert).

This stage of research consists of making *lu'at* as shown in Figure 1. Fermented *lu'at* is sampled for the calculation of total lactic acid bacteria and pH values during *lu'at* fermentation. In making *lu'at*, cayenne pepper, lime, and basil are each sorted, washed, and then drained until dry. Cayenne pepper is mashed using a chopper. Lime is split, seeded, and thinly sliced (2-3 mm). Basil is dried in the afternoon and only the leaves are taken. All of these ingredients are then weighed according to the formulation (150 grams of cayenne pepper, 10 grams of lime,

and 5 grams of basil leaves) and put in a bowl with the addition of table salt (*Kapal Api*) with salt content of 0%, 5%, and 10% w/w, then mixed. This *lu'at* is then put in a jar and sealed tightly, then fermented at room temperature for 0, 7, and 14 days under hygienic conditions to prevent contamination.

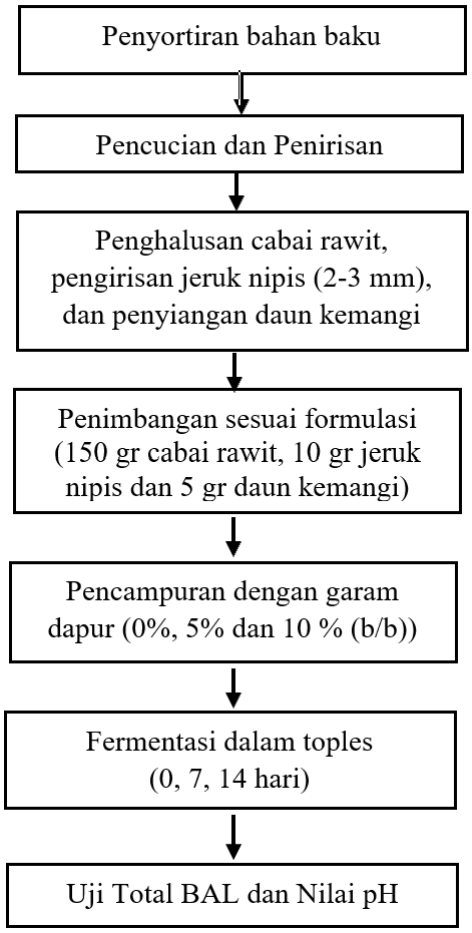

Figure 1. Flow chart of making Lu'at.

The total calculation of Lactic Acid Bacteria (LAB) using the Total Plate Count (TPC) method using MRS agar medium. Ten grams of *lu'at* samples were prepared and serial dilution was carried out by homogenizing the sample in 90 mL of 0.1% buffered peptone water (Oxoid) (10^-1 dilution) in an Erlenmeyer flask, taking 1 mL of sample from dilution 10^-1 and transferring it to the next dilution containing 9 mL of 0.1% buffered peptone water and homogenized with vortex, and continued in stages until dilution 10^-6. One mL of sample from each dilution was taken and spread onto MRS agar medium using the spread plate method and then spread evenly using a Drigalski spreader. The plates were wrapped and incubated upside down at 37°C for 48 hours.

For pH value measurement, 10 grams of sample were taken aseptically, mixed with 10 mL of distilled water, and measured using a calibrated pH meter (PHS-3C).

The data from the study were analyzed using Analysis of Variance (ANOVA) at a significance level of 0.05 using Minitab Statistical Software 22, and if there were significant differences, follow-up testing was performed using the Tukey test.

RESULTS AND DISCUSSION

Total Lactic Acid Bacteria (BAL)

Total testing of lactic acid bacteria (BAL) is an important parameter in fermented foods that aims to determine the presence and growth of lactic acid bacteria in the food. Lactic acid bacteria in fermented foods can come from the natural microflora of the food Putri et al., (2024) or from pure cultures added to fermented foods (Seveline et al., 2021). In this study, the total lactic acid bacteria were calculated in fermented lu'at with different salt levels and fermentation durations. The fermented lu'at can be seen in figure 2.

(a) (b) (c)

Figure 2. Lu'at (a) salt content 0%, (b) salt content 5%, (c) salt content 10%

The average total lactic acid bacteria in lu'at fermentation with different salt levels and fermentation lengths can be seen in table 1. The average total lactic acid bacteria in this lu'at fermentation ranges from 105 to 107 CFU/g. The highest average total BAL in lu'at fermentation was found in treatment with a salt content of 5% with a storage period of 7 days (4.3 x 107 CFU/g). Meanwhile, the total BAL is lowest in lu'at fermentation with a salt content

of 10% with a fermentation duration of 0 days. The results of ANOVA showed that treatment with different salt levels had a significantly different effect ($p<0.05$) on bacterial growth, but the treatment duration of fermentation and the interaction between salt content and fermentation time did not have a significant difference ($p>0.05$). The results of the Turkey Test showed that treatment with a salt content of 5% had a significantly different effect from the treatment of 10% salt content on the growth of lactic acid bacteria but the effect was not significantly different from the treatment of salt content of 0%.

Table 1. Total Average (BAL) (CFU/g)

Salt Levels	Fermentation Time		
	0 Days	**7 Days**	**14 Days**
0%	1.3 x 107 ^{AB}	1.2 x 107 ^{AB}	9.8 x 106 ^{AB}
5%	7.5 x 106A	4.3 x 107 ^{A}	1.8 x 107 ^{A}
10%	**7.9 x 104 ^{W}**	**7.1 x 105 ^{W}**	**1.2 x 106 ^{B}**

Remarks: Numbers followed by different letters in the same column show a real difference ($p < 0.05$) based on the Tukey Test.

This study shows that the high salt content in lu'at fermentation has the lowest total BAL even though it has increased until day 14. This is suspected because the high salt content in lu'at is not optimal in supporting the growth of lactic acid bacteria at the beginning of fermentation. Salt content can function to select the types of microbes that can grow on food depending on the resistance of those microbes to salt. BAL growth in fermented vegetables is inhibited at high salt concentrations (>5%) influenced by osmotic pressure, metabolism and bacterial enzyme activity. However, at low to optimal salt levels (2-3%) BAL growth becomes rapid (Anggraeni et al., 2021). Salt can selectively inhibit or support the growth of bacteria. A study on the fermentation of 6 chili cultivars over 21 days showed that there was a total lactic acid bacteria of 3.8 x 106 - 6.2x 108 CFU/g which increased from 50-3,700 CFU/g in raw chili peppers [24]. A study on the fermentation of salted chili sauce showed that the total number of lacic acid bacteria decreased in treatment with higher salt levels. The results of this study showed that treatment with a salt content of 15% and a fermentation period of 5 days had a total of 7x1011 CFU/g of lactic acid bacteria (Juliarsi & Werdiningsih, 2018).

In addition, tolerance to salt is also affected by the type of BAL. Good conditions for BAL can be created with optimal salt composition (Matti et al., 2021). BAL can grow in acidic conditions (acidophyll), produce lactic acid and cause a decrease in pH value as fermentation takes place (Nurfuzianti, 2021). In cheese fermentation with different types of BAL, most types of BAL are able to show tolerance to a salt content of 6%, but only a few are able to survive at a salt content of 10%, namely *L. delbruecki* (P14, P38*), L. rhamnosus* (P50), and *L. plantarum* (Q3C4) (Nicosia et al., 2023). However, fermentation occurs spontaneously without the addition of microorganism cultures, so it is not known what type of BAL is able to survive the salt level conditions in the treatment. Thus, it takes a sufficient salt content and storage time to help the growth of BAL in lu'at fermentation. In addition, it is also necessary to know the type of BAL that plays a role in the fermentation of Lu'at which can grow at varying salt levels.

pH value

The pH values of Lu'at fermentation with different salt content and storage times are shown in table 2. Lu'at fermentation with different salt levels and fermentation durations has an average pH value of 3.57 – 3.98. The ANOVA results showed that there was no significant difference between pH values across all treatments and interactions between treatments ($p>0.05$). In general, there is a decrease in pH in Lu'at fermentation. Lu'at fermentation with a salt content of 5% and a fermentation period of 7 days showed the lowest pH (3.57). This is comparable to the highest BAL growth in the treatment (4.3 x 107 CFU/g). However, at the end of fermentation, there is an increase in the pH value to 3.94. This shows that the salt content and the fermentation time have no effect on the pH value of fermentation lu'at.

Table 2. Average pH Value

Salt Levels	Fermentation Time		
	0 Days	**7 Days**	**14 Days**
0%	3.98 ^{A}	3.87 ^{A}	3.77 ^{A}
5%	3.88 ^{A}	3.57 ^{A}	3.94 ^{A}
10%	**3.77** ^{A}	**3.74** ^{A}	**3.68** ^{A}

Remarks: Numbers followed by different letters in the same column show a real difference ($p < 0.05$) based on the Tukey Test.

A decrease in pH value is thought to be beneficial for the preservation of chili peppers through the fermentation process. A decrease in pH values is an indication of the success of fermentation to prevent the growth of rotting bacteria and pathogens (Matti et al., 2021). A decrease in the pH value of fermented products can occur due to the addition of salt (Handayani, 2023). The pH value is not only affected by the growth of lactic acid, but also by the moisture content (Barani et al., 2023). In addition, the presence of lime in lu'at also plays a role in making the acidic condition of this chili sauce. The increasingly acidic pH value causes lactic acid bacteria to survive and become a condition that supports the activity of BAL to produce lactic acid compounds from the breakdown of sugars (Handayani, 2023).

CONCLUSION

Based on the results of the research conducted, it can be concluded that salt content has a significant effect on the growth of lactic acid bacteria in *lu'at* fermentation. In addition, the results of this study also show that there is no significant difference in the total lactic acid bacteria and the pH value in fermentation based on the length of fermentation time. *Lu'at* fermentation treatment with a salt content of 5% resulted in the highest total lactic acid bacteria. Treatment with 5% salt content and a fermentation time of 7 days also resulted in a total lactic acid bacteria of 4.3 × 10^7 CFU/g and the lowest pH value (3.57). Based on the results of this study, it is necessary to conduct further research on fermentation of *lu'at* with different formulations, isolation and identification of microorganisms that play a role in fermentation of *lu'at*, as well as physicochemical quality analysis in fermentation of *lu'at*. Additionally, future studies should investigate the sensory characteristics and shelf-life stability of *lu'at* under optimal fermentation conditions, explore the antimicrobial properties and probiotic potential

of the isolated lactic acid bacteria, examine the nutritional composition and bioactive compounds present in fermented *lu'at*, and develop standardized protocols for commercial-scale production to support the preservation and commercialization of this traditional Indonesian fermented food product.

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