



# Isolation and Total Lactic Acid Bacteria from Sauerkraut with the Addition of Chili (*Capsicum Annum L.*)

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**Abstract.** Sauerkraut (suerkhol) is the result of lactic acid fermentation of chopped white cabbage with a length of about 20 cm and a width of 2 mm to 5 mm. Based on research that has been done successfully isolated 17 lactic acid bacteria isolates from Sauerkraut. Chili has a unique taste and contains alkaloid compounds such as capsaicin, with this content it is hoped that chili can be a source of nutrition for lactic acid bacteria (LAB). Lactic acid bacteria have an essential role in almost all fermentation processes of food and beverages, such as dairy products and probiotic drinks such as yogurt. Then this research aims to Isolate preliminary counting of total LAB from Sauerkraut with the addition of chili. Isolation of LAB from Sauerkraut was isolated by pour plate method using MRSA (Man Ragosa Sharpe Agar) selective medium. This study was an experimental study using a completely randomized design (CRD) with five additional treatments of chili slices (control, 5%, 10%, 15%, and 20%) and three replications. Samples according to the treatment were fermented for four days. The total number of bacteria was observed every day with different samples. Bacteria were isolated and grown on MRSA media. Data were analyzed by analysis of variance (ANOVA) at the 5% level and DMRT follow-up test. The total amount of LAB in sauerkraut was affected by the addition of chili slices. Total LAB highest found in the addition of sliced chili 20% ( $65.18 \times 10^8$  CFU/ml) and the lowest was found in controls ( $0.01 \times 10^8$  CFU/ml). Maximal P growth LAB in the process of fermentation of sauerkraut in the gain on the third day of fermentation for all treatments. The provision of chili slices can increase the total amount of LAB in sauerkraut fermentation.

**Keywords:** LAB · Chili · Sauerkraut · Fermentation

## 1 Introduction

The Sauerkraut is often referred to internationally as sour cabbage which originates from Germany. In Indonesia, the sauerkraut is a product of lactic acid fermentation of white cabbage chunks size of the width 2–5 mm and length of about 20 cm [1].

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M. Fadilah et al. (Eds.): IcoBioSE 2021, ABR 32, pp. 290–296, 2023.

[https://doi.org/10.2991/978-94-6463-166-1\\_38](https://doi.org/10.2991/978-94-6463-166-1_38)

The microorganisms used in fermentation processes for sauerkraut are *Lactobacillus mesenteroides* and *Lactobacillus plantarum*. The Sauerkraut is made by adding salt (NaCl) [2]. In making this sauerkraut only use microorganisms that already exist in the cabbage, namely lactic acid bacteria with the addition of salt to help lactic acid bacteria work because salt will remove nutrient liquid from the cabbage so that lactic acid bacteria are easier in the fermentation process. In addition, salt can also be used as an antimicrobial [3].

Chili plants (*Capsicum annum* L.) can be processed into many products as the main seasonings. This chili is very popular with the public, especially in Indonesia, because of its unique taste [4], so it has a high selling value. The C ignorant contains 1.0 g protein, 0.3 g fat, 7.3 g carbohydrates, 29 m g of calcium, phosphorus, iron, 18 mg of vitamin C and 0.05 mg of vitamin B1, as well as the alkaloid compounds such as capsaicin [5]. The content of chili can be a source of nutrients for bacteria acid lactate in vegetable fermentation processes and also be giving flavor to the product of fermentation.

Lactic acid bacteria is a bacterium with the following criteria: the ability to ferment carbohydrates, gram-positive bacteria, bacillus-shaped, not spore, and facultative [6]. This bacterium is able to produce lactic acid, hydrogen peroxide, and antimicrobial and metabolic outcomes e others are beneficial for the body of humans [7]. The purpose of this study was to isolate and calculate the total Lactic Acid Bacteria from Sauerkraut with the addition of Chili (*Capsicum annum* L).

## 2 Materials and Methods

### 2.1 Tools and Materials

The tools used in this study were analytical scales, jars, knives, cutting boards, test tubes, test tube racks, stirring rods, dropper, bunsen, electric stove, ose needle, Erlenmeyer, autoclave, incubator, vortex, Petri dish, funnel, glass measuring.

The materials used in this study were fresh cabbage and chili obtained from the Padang market and thinly sliced, salt, medium mann rogose Sharpe agar (MRS A), sterile distilled water, physiological NaCl, 70% alcohol and label paper.

### 2.2 Research Design

This research design used RAL which consisted of five treatments with three replications. The treatment given to fermented cabbage with the addition of chili slices was as follows:

- K = Cabbage 100g + 2.5% salt.
- A = Cabbage 100g + salt 2.5% + chili 5%
- B = Cabbage 100g + salt 2.5% + chili 10%
- C = Cabbage 100g + salt 2.5% + chili 15%
- D = Cabbage 100g + salt 2.5% + chili 20%

## 2.3 Research Procedure

### 2.3.1 Preparation Stage

#### Tool Sterilization

All media and tools made of glass are washed and sterilized before use. Sterilization process of all mediums and tools using an *autoclave* at a temperature of 121 °C and a pressure of 15 psi (*Pounds Per Aquare Inchi*) within 15 min. Tools made of materials other than glass are sterilized using 70% alcohol, such as tweezers and *ose* needles.

#### MRSA Media Production

The previous use of MRSA media was carried out by weighing the material first, namely 6.2 g of MRSA powder media dissolved in an *Erlenmeyer* using 100 ml of distilled water while heating and stirring until the solution was homogeneous. After that, the finished medium was sterilized for 15 min using an *autoclave* at a temperature of 121 °C and a pressure of 15 psi.

## 2.4 Implementation Stage

### 2.4.1 Sample Setup

Firstly, the tools and materials for making sauerkraut must be prepared. The next stage is the selection of good cabbage and chili by paying attention to the level of freshness and decay of the cabbage and chili that will be used. After the cabbage and chili of good quality are obtained, the processing is carried out by washing the cabbage and chili first and discarding the parts that are not used. After that, slice the cabbage and chili with a thickness of approximately 0.5 cm. Then mix the thinly sliced cabbage with 2.5% salt and sliced chili according to the treatment. After that, put the mixture of cabbage, salt, and chili slices into the jar by pressing until the cabbage water comes out covering the surface of the cabbage slices and making sure there are no bubbles in the jar, then close the jar tightly and let it rest for 4 days for the fermentation process.

### 2.4.2 Isolation of Lactic Acid Bacteria

Isolation of LAB was done by *the ur plate* method. A total of 1 g of sample was put into a test tube that was ready containing physiological NaCl as a diluent solution of 9 ml so that a dilution of  $10^{-1}$  was obtained. The dilution is carried out from  $10^{-1}$  to  $10^{-8}$  by taking 1 ml of the solution from the previous dilution. After that as much as 1 ml of each sample solution that has been diluted starting from a dilution of  $10^{-3}$  to  $10^{-8}$  is taken and put into a sterile petri dish, then pour 20 ml of MRSA media evenly to form a Fig. 8. Wait for the media MRSA hardened, wrapped in *Petrising wrapping*, and incubated for  $1 \times 24$  hours at room temperature with an inverted position for each treatment on the total calculation of lactic acid bacteria and for the fermentation time carried out in the same way with the calculation of total LAB which was carried out every day for  $1 \times 24$  fermentation,  $2 \times 24$ ,  $3 \times 24$  and  $4 \times 24$  hours.

### 2.4.3 Calculation of Total LAB

Lactic acid bacteria (LAB) which were counted on MRSA media were all growing colonies with the number of 25 to 250 colonies expressed in cfu/g. The number of LAB can be calculated using the following formula:

$$\text{Num. bacteria} = \text{num. of colonies} \times \frac{1}{\text{Diluent factor}}$$

### 2.5 Data Analysis Technique

The data that has been obtained were analyzed using the ANOVA (Analysis of Variance) test and if significantly different results were obtained, the test was continued by using a significant difference test with a significance level of 5% using the Duncans Multiple Range Test (DMRT) method.

## 3 Results and Discussion

### 3.1 Total Lactic Acid Bacteria LAB

The total results of LAB contained in cabbage sauerkraut within four days of fermentation can be seen in Table 1.

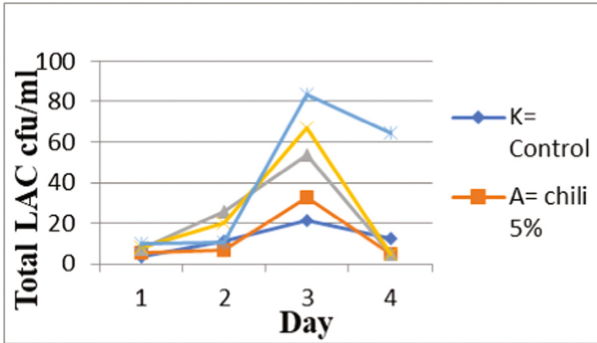
From Table 1, it can be seen that the addition of chili (*Capsicum annum L.*) in various amounts, namely 5%, 10%, 15%, and 20% within 4 days of fermentation affects the total lactic acid bacteria that grow on sauerkraut so as to produce a total of lactic acid bacteria that different. From the results of the analysis of variance, it is known that  $F_{\text{count}} > F_{\text{table}}$  so that  $H_0$  rejected at a significant level of 0.05. Therefore, it can be said that the addition of chili has an effect on the total lactic acid bacteria, the higher the number of chili slices added, the higher the total yield of lactic acid bacteria that grows

Fermentation is a process of chemical change in an organic substrate through the activity of enzymes produced by microorganisms [8]. One of the important factors in the fermentation process is the growth of microorganisms. This total LAB observation is

**Table 1.** Table of Average Total LAB on Cabbage Fermentation with the Addition of Chili Slices

Treatment	Average
K	0.010 <sup>a</sup> cfu/ml
A	4.083 <sup>a</sup> cfu/ml
D	4,696 <sup>a</sup> cfu/ml
C	27.296 <sup>b</sup> cfu/ml
B	65,176 <sup>c</sup> cfu/ml

Note: Figures are d follow the letter that the same is not significantly different, at the level of 5% by DMRT



**Fig. 1.** Average Growth of Lactic Acid Bacteria Every Day in Fermented Cabbage with Addition Sliced Chilli

expressed in total microbial units, namely colony-forming units per milliliter or abbreviated as cfu/ml [9]. Making sauerkraut with the addition of chili slices is a spontaneous natural fermentation without the addition of a starter or other microbes from outside [10].

### 3.2 Fermentation Time

The results of fermentation time on total LAB on cabbage fermentation in the manufacture of sauerkraut were carried out once every 24 h for 96 h or once a day for 4 days. The total LAB growing on sauerkraut with added chili slices every day for each treatment can be seen in Fig. 1.

The addition of chili slices with different amounts affects the total LAB, resulting in a different total LAB every day. Total LAB tends to increase during the fermentation of one day to three days. However, on the fourth day of fermentation, the total LAB that grew tended to decrease from the previous day. In addition to influencing the growth of LAB, chili also affects sauerkraut in terms of taste, color, aroma and texture. From the research that has been done obtained results, the treatment of fermentation on the third day is the best treatment or fermentation time to ferment sauerkraut cabbage for total LAB are grown on the third day of fermentation is the highest total LAB. This shows that on the third day LAB growth enters a stationary phase according to the bacterial growth curve, namely LAB growth is at its maximum point of growth which will then be followed by a death phase [11].

## 4 Conclusion

Based on the research that has been done, namely about the effect of adding chilies to the total LAB in cabbage sauerkraut, it can be concluded that the addition of chili slices in fermented cabbage can increase the total LAB in cabbage sauerkraut the highest total LAB is with the addition of 20% chili slices. The cabbage fermentation time affects the total LAB, the best fermentation time for LAB growth is three days.

**Acknowledgments.** The author would like to thank LP2M Universitas Negeri Padang which has funded this research with contract No: 867/ UN3513/LT/2021. The Dean of the Faculty of Mathematics and Natural Sciences, the Head of the Department of Biology and his staff, the Head of the Biology Laboratory, and his staff for permission to carry out the research, and all parties who have assisted in the implementation of this research.

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