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The Effect of Additional Chili (*Capsicum annum*) and Sugar on Total Lactic Acid Bacteria (LAB) from Sauerkraut

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Abstract: Sauerkraut is a typical German food made from thinly sliced cabbage. Sauerkraut is a fermeted product that can give a distinctive taste. Sugar is an ingredient that can be added in, that sugar can trigger the growth of lactic acid bacteria. Indonesian people like food with a spicy sensation from chilies, chillies here contain vitamin C which functions as an antioxidant that is good for the body and is able to increase endurance. This study aims to calculate the total lactic acid bacteria contained in sauerkraut with the addition of chilies and sugar. Dilution of 1 ml sample into 9 ml physiological NaCl solution from 10^{-1} to 10^{-8} dilutions was carried out, then inoculated into MRS Agar using the pour plate method. Incubated in room temperature for 48 hours. The growing colonies of lactic acid bacteria were then observed based on colony morphology and counting the total growing lactic acid bacteria. The results of the research can be concluded that there was an increase in the number of lactic acid bacteria in sauerkraut with the addition of chili and 10% granulated sugar with a bacterial density average is 88.667 x 10^8 CFU/mL.

Keywords: Chili; Lactic Acid Bacteria; Sauerkraut; Sugar

Introduction

Sauerkraut (sour cabbage) is a typical German food made from finely chopped and fermented cabbage. Fresh vegetables and fruits are minimally processed and especially have a short shelf life due to rapid microbial decay, and, in some cases, against contamination by pathogens (Di Cagno et al., 2013), so need to picklish for longer time use. Sauerkraut is a cabbage product produced through fermentation by microbial succession of lactic acid bacteria and is a potential source of probiotics. Sauerkraut is useful as a catalyst for the digestive process in the body because sauerkraut contains lots of probiotic bacteria (good bacteria), namely Lactobacillus plantarum which can release gas in the stomach and overcome defecation (Koswara, 2013). Lactic acid bacteria are produced by fermenting the sugar contained in cabbage. According to Rahmah et al. (2014) cabbage contains a lot of air which causes the plant to rot easily and not last long in storage. For this reason, a legal process can be carried out so that cabbage products can last a long time.

Various additional ingredients are used in making fermented vegetables (pickles), such as salt, vinegar, spices such as chili powder, turmeric powder, fenugreek seeds, asafoetida, sugar, jaggery and oil (Monika et al., 2017). Meanwhile, sauerkraut is a cabbage product that is fermented using a certain concentration of salt. The addition of salt during fermentation can trigger the development of lactic acid bacteria, especially Leuconostoc and Lactobacillus species, which are able to convert sugar in vegetables into lactic acid, thereby limiting the growth of pathogenic organisms (Utama et al., 2018). Salt contains nutrients that act as an ideal substrate for the growth of lactic acid bacteria. Lactic Acid Bacteria (LAB) are a group of bacteria that are able to convert carbohydrates (glucose) and produce lactic acid as the main product in fermentation (Masood et al., 2011).

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There are many factors can influence LAB growth. The fermentation process carried out by LAB is influenced by several environmental factors such as temperature and pH. Media with high sugar concentrations show increased sugar consumption which indicates increased LAB growth. In fermented products such as kimchi, the addition of spices in the form of chilies can lower the pH, increase acidity, and improve the taste of the product (Jeong et al., 2013). Apart from that, the addition of 10% ground chili can increase LAB growth (Normayanti et al., 2020).

Sauerkraut is a natural bacterial fermentation with the help of 2.5% salt. The addition of salt causes the growth of lactic acid bacteria and limits the growth of gram-negative bacteria. The addition of salt is necessary for the development of anaerobic conditions during fermentation and for inhibiting the growth of spoilage microorganisms and activity of endogenous pectinolytic enzymes responsible for cabbage softening (Peñas et al., 2017). Sauerkraut with the addition of cayenne pepper can provide many health benefits and can add economic value to the point that it is ignored by the fermentation process (Fevria & Hartanto, 2019). Chili is a type of plant that has high economic value. Chili contains vitamin C as a nutrient which acts as an antioxidant and is effective in dealing with free radicals which can damage cells or tissue, including protecting the lens from oxidative damage due to radiation (Rosmainar et al., 2018).

Pickled cabbage, which is made by adding starter cultures of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*, has a lower pH value, acetic acid content and total sugar, but higher titratable acidity and total lactic acid bacteria (LAB) compared with ordinary cabbage pickle (Pato et al., 2019). Sugar (sucrose) is a disaccharide of glucose and fructose compared to maltose and ketose which are also disaccharides. Sucrose does not contain free anomeric carbon atoms. Sugar can play a role in accelerating the growth of lactic acid bacteria in the fermentation process (Zulfahmi & Nirmagustina, 2012).

Based on this explanation, it is known that the addition of additional ingredients and spices has an effect on the fermentation process and growth of LAB. For this reason, this research was carried out to see how LAB grows in sauerkraut that is added with sugar and chili at various concentrations.

Method

This type of research is descriptive. This research was conducted in the research laboratory of the Biology Department, FMIPA UNP. This research used a completely randomized design with 5 treatments and 3 repetitions. Treatments A (5% sugar + chili), B (10% sugar + 10% chili), C (10% sugar + 5% chili), D (5% sugar + 10% chili), and E (control). The tools used in this research are petridish, beaker glass, test tube and rack, stirring rod, spatula, measuring cup, electric stove, Bunsen burner, vortex, digital scale, micropipette, tips, erlenmeyer, autoclave, label paper, marker pen, plastic wrap, measuring cup, aluminum foil, basin, knife. The ingredients needed are cabbage, table salt, red chili, water, MRS agar medium, sterile distilled water, physiological NaCl.

Making Sauerkraut with Added Chili and Sugar

The cabbage and red pepper should be cleaned under running water. Then, slice them to approximately 0.5 cm thickness. Weigh 100 g of cabbage and 10 g of chili and sugar each. After weighing the cabbage, mix it with 2.5 g of salt until it wilts and releases water. Add the chili and sugar, and stir until well blended. Take a mixing jar and put the mixture in it, pressing it down until the entire surface is covered with water and no air bubbles remain. Close the jar tightly and wrap it air-tight. Let it ferment at room temperature for four days. Afterward, analyze the shape and aroma of the fermented mixture.

Making MRSA Media (DeMan Rogosa Sharpe Agar)

The use of MRSA (DeMan Rogosa Sharpe Agar) as a growing medium for lactic acid bacteria includes several steps, including: 6.715 g of MRSA powder is weighed and then dissolved in 100 ml of distilled water in an Erlenmeyer. Then dissolved and heated on a hot plate until boiling. Then sterilized in an autoclave.

Calculation of Lactic Acid Bacteria

A total of 1 ml of the sample was put in a test tube containing 9 ml of physiological NaCl diluent to obtain a dilution of 10-1. Furthermore, as much as 100 microlite suspension from dilutions 10-6, 10-7, 10-8 was taken and put in a sterile petridish, and then 10 ml of MRSA medium was poured and shaken evenly. After the agar dries the petridish is wrapped in warpping and incubated. in an inverted position at room temperature for 2 x 24 hours. The number of colonies used to calculate the total number of lactic acid bacteria is 25-250 and is expressed in CFU/mL using Equation:

$$\text{Total bacteria} = \text{Total colonies} \times \frac{1}{\text{dilution factor}} \quad (1)$$

Result and Discussion

This research used samples of sauerkraut originating from cabbage that was fermented itself for 4 days, with 4 treatments and 1 control. Then LAB was isolated from sauerkraut and then the average number of bacteria was calculated. It can be seen in Figure 1.

The highest average number of bacteria in treatment B was significantly different from the control, followed by treatments C and D. However, it was not significantly different from treatment A. The average results showed that the addition of different chilies and

sugar affected the number of LAB in sauerkraut. . The addition of 10% chili and a sugar concentration of 10% produced the highest number of lactic acid bacteria, where sugar is a nutrient needed by bacteria to grow. This was then followed by the BAL C sample which received an additional concentration of 10% sugar and 5% chili. Based on this, the presence of sugar has a big influence on LAB growth, where sugar is a carbon source which becomes a nutrient for LAB growth. Concentration of sugars may also influence the growth and functionality of LAB (Saeed & Salam, 2013).

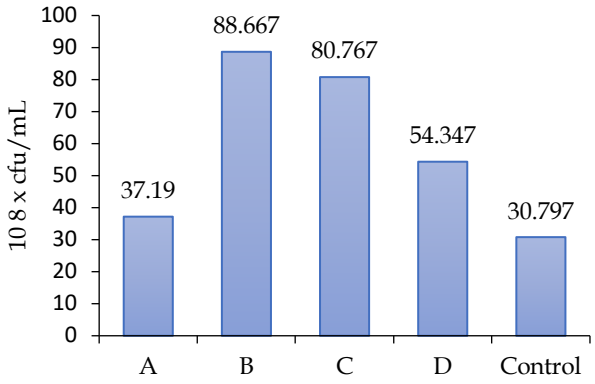

Figure 1. Number of Lactic Acid Bacteria

Glucose fermentation under standard conditions divides LAB species into two groups, namely, homofermentatives which convert glucose almost quantitatively into lactic acid and heterofermentatives, fermenting glucose into lactic acid, acetic acid and CO (Lahtinen et al., 2011). Apart from that, different types of sugar sources such as glucose, maltose, mannose and galactose are also preferred by LAB as a carbon source, these sugars can be used as alternative nutritional sources other than those provided by MRS medium (Petrut et al., 2019).

A carbon source (in this case sugar) is needed to produce LAB. Differences in sugar concentration will affect the presence of LAB. If it is too low it will reduce BAL growth, while if it is too high and excessive it will inhibit BAL growth. Which tested the effect of various sugar concentrations on LAB, namely, at a concentration of 40g/L LAB experienced a decrease in growth. Meanwhile, the optimal concentration is 80g/L. And if the concentration is increased to 100g/L it will also cause a decrease in LAB growth (Hassan et al., 2015).

Table 1. Total Lactic Acid Bacteria (CFU/mL)

Sample	I (x 10^8)	II (x 10^8)	III (x 10^8)	Total (x 10^8)
Control	32.88	30.08	29.43	92.39
A (S5%+C5%)	37.57	35.39	38.61	111.57
B (S10%+C10%)	98.59	85.49	81.93	2668
C (S10%+C5%)	64.67	73.12	104.51	242.3
D (S5%+C10%)	56.3	56.77	49.97	163.04

From table 1. It can be seen that apart from the influence of sugar, chili also has quite an influence on LAB growth, although not too significantly. This is proven by the difference in total bacteria in sample A (sugar 5% + chili 5%) with the addition of 5% chili in sample D which did not show too much increase in the number of LAB, which was originally 111.57x108 after adding chili only increased to 167.04x108. This shows that during this fermentation the chilies had too much influence.

Fermentation with chilies will involve many microorganisms in the spontaneous fermentation process which allows the production of aerobic bacteria (Cai et al., 2021). In research by An et al. (2021), pickle samples that used the addition of chili oil had fewer colonies than other pickles. This is thought to be because the addition of chili or chili powder can inhibit microbial activity due to the capsaicin it produces (Jeong et al., 2013). Apart from that, in Asian culture, the addition of chilies is often preferred to add flavor and aroma to pickled products.

Sauerkraut with the addition of chili is a fermentation that occurs naturally without the addition of external microbes and occurs naturally with the help of native microflora (Fevria & Hartanto, 2020). Fermentation is assisted by gram with a concentration of 2.5%, where salt with a high concentration can cause obstruction of the fermentation process, while salt with a concentration of less than 2.5% can cause the growth of proteolytic and celluliotic bacteria which disrupt the fermentation process (Utama et al., 2018). The cabbage shredded and added the salt, sugar and chili or pepper. When shredded cabbage is pressed during fermentation, the concentration of oxygen decreases in the vessel, leading to a reduction of dominant aerobic bacterial communities. These anaerobic conditions, however, favor the growth of LAB populations. It is known that during cabbage fermentation a rapid turnover of different heterofermentative and homofermentative LAB species occurs due to the modification of ecological conditions throughout the process. Fermentation is initiated by heterofermentative LAB, mainly Leuconostoc mesenteroides (Peñas et al., 2017). The use of mineral salt in combination with Leuconostoc mesenteroides resulted in an especially mild tasting sauerkraut juice with good sensory and microbiological quality (Wiander & Ryhänen, 2005).

MRSA is an selected medium used to isolate lactic acid bacteria. The use of isolation media for bacterial isolation is to facilitate the growth of certain microbial strains and inhibit the growth of other microbial strains. MRSA medium contains polysorbate, acetate, magnesium and manganese which are growth factors for Lactobacillus, Streptococcus, Pediococcus and Leuconostoc. Due to the nature of releasing entire

colonies of lactic acid bacteria, several strains can grow on this medium (Mohamed et al., 2016).

Exploitation of bacteriocinogenic lactic acid bacteria on common spoilage and poisoning of raw vegetables and fruits was largely carried out. Several examples of bio-preservation of freshcut salads and other vegetables (e.g., apples and lettuce) from common spoilage (yeasts and moulds) and poisoning (*Listeria innocua*, *Listeria monocytogenes* and *Escherichia coli*) microorganisms were reported (Fan & Hansen, 2012). Various types of LAB can be produced by various biological or animal products. Such as fermenting several fruits or vegetables, the traditional Kalimantan fruit Tapos (Rahayu & Setiadi, 2023), cocoa (Tsaaqifah et al., 2023) and also pineapple with rabbitfish (Mahrus et al., 2023). Bacteria mainly populate the biota of vegetables, especially the aerobes (pseudomonads, enterobacteria and coryneforms) (Schneider, 1988).

Calculation of the number of lactic acid bacteria colonies was carried out starting from dilutions of 10 -6 to 10 -8. The results of conservation of the number of lactic acid bacteria colonies from sauerkraut with the addition of chili and sugar can be seen in Figure 2. It can be seen that adding 10% of chili and granulated sugar can increase the growth of lactic acid bacteria during the 4 day fermentation time. The comparison between control, 5% sugar and 10% sugar is clearly different. Sugar acts as a nutrient for the development of bacteria, the higher the concentration, the greater the number of bacterial colonies (Fevria et al., 2020).

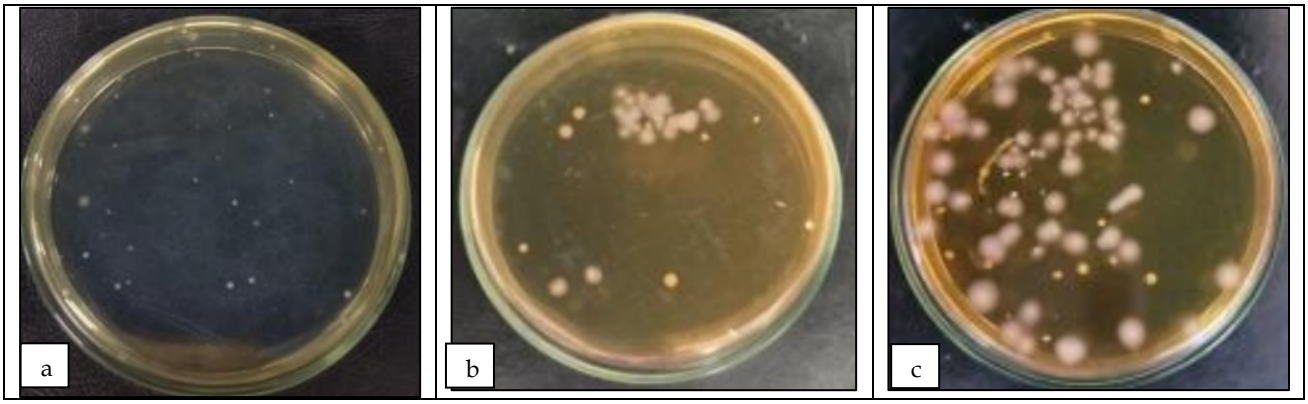

Figure 2. Number of Lactic Acid Bacteria. a (control), b (sugar 5%+chili 5%), c (sugar 10%+chili 10%).

Many things can influence fermentation results and the presence of LAB. Beside the concentration of sugar and chili addition. In addition to nutritional supplements and the provision of additional ingredients, environmental factors, treatments such as temperature and pH also influence the LAB produced (Tsaaqifah et al., 2023). Available sources of energy, carbon and nitrogen that may influence LAB growth and metabolism (Saeed & Salam, 2013). Various factors can be optimized by varying various parameters including salt solution concentration and pH value, product texture and sensory characteristics in order to improve the quality of fermentation and LAB (Hien et al., 2022). Different types of food products contain different water contents. If the food contains sufficient or high water content then dry salt is added to the preserving mixture. Sometimes sugar is also added as a preservative to increase the fermentation rate and add sweetness to pickled foods. Lactobacillus Bacilli, a type of Lactic Acid Bacteria (LAB), is used for the natural fermentation process (Krishnan et al., 2014). When favourable conditions of anaerobiosis, water activity, saltconcentration and temperature occur, raw vegetables and fruitsmay be subjected to spontaneous lactic acid fermentation. In somecases, the alcoholic fermentation takes place concomitantly (Di Cagno et al., 2013).

The addition of appropriate amounts of preservatives such as salt, acid, and sugar reduces the growth of undesirable microorganisms. Along with preservatives, physical conditions such as temperature, acidity, and time are necessary for proper fermentation to occur. The temperature should be maintained between 15 to 25°C and the pH around or less than 4.5 to allow proper fermentation to occur. Temperatures over 25°C can cause rot. As fermentation requires such a low pH, some bacteria grow in such conditions (Patel, 2019).

In this study, all samples were added to the same amount of salt. The addition of salt in fermenting vegetables functions to preserve and add flavor to sauerkraut. Salt plays a major role in the fermentation process and functions to remove nutrients and water from the vegetables used as ingredients, while salt is also used as a substrate for LAB growth (Hassan & Sarfraz, 2018). The presence of salt and acetic acid in pickle fermentation has been proven to significantly eliminate contamination from pathogenic microorganisms and foodborne bacteria (Aljahani, 2020). Generally, some pickle products use salt levels of 15-20% to support LAB growth (Hassan & Sarfraz, 2018). However, if the salt

level is too high it can have a bad impact on health, such as increasing the risk of heart disease and hypertension (Singh et al., 2017). Only a limited number of LAB genera can grow in high salinity conditions, thereby suppressing the dominance of LAB in the salt stock preparation process (Sawada et al., 2021).

Fermentation time can also influence the presence of LAB. Heterofermentative LAB can reach 9 log10 CFU/mL during the first week of fermentation and then the number decreases because it replaced by homofermentative microorganisms that are more tolerant to acid (Breidt et al., 2013). The fermentation process can increase the effect on the level of bioactive components and antioxidant capacity of vegetables, the length of the fermentation process can influence the antioxidant content of pickles, and generally can be maintained during the 30 day fermentation process (Sayin & Alkan, 2015).

Acidity determines the ability of microorganisms to grow in fermented products. To prevent the growth of microorganisms or the acidity of certain foods should be increased. Acidity must be maintained in such a way as to prevent bacterial growth and at the same time be safe for human consumption (Patel, 2019). Fermentation of salt also affects the pH of the fermented product, chili peppers fermented using aged brine exhibited a significantly lower pH value and higher organic acid content than that fermented using fresh brine (Zhang et al., 2022).

Conclusion

Based on observations of colony morphology and the results of calculations of lactic acid bacteria growing on MRS Agar media, it can be concluded that the amount of LAB in sauerkraut with the addition of chili and sugar 10% can increase LAB grow, and the calculation of LAB colony is 111.5×10^{8} CFU/mL.

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Author Contributions

Conceptualization, V. R. F, M. I, M. C, A. A , E.; Methodology, R. F, M. I,.; Validation, M. C, A. A.; Formal analysis, R. F, M. I.; Investigation, R. F, M. I; resources, R. F, M. I.; data curation, R. F, M. I.: writing—original draft preparation, M. I and R. F.; writing—review and editing, V. R. F, M. I,.: visualization, and R. F.and M. I. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The author declares that there are no conflicts of interest in this published work.

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