**EFFECT of SALT CONCENTRATION AND FERMENTATION TIME to LACTIC ACID CONCENTRATION in RADISH *(Raphanus sativus L)* PICKLE MANUFACTURING**

**ARTICLE**

**Trisna Megawati**

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**DEPARTEMENT OF FOOD TECHNOLOGY**

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**EFFECT of SALT CONCENTRATION AND FERMENTATION TIME to LACTIC ACID CONCENTRATION in RADISH *(Raphanus sativus L)* PICKLE MANUFACTURING**

Dede Zainal Arief

Sumartini

Trisna Megawati

Departement of Food Technology, Engeenering Faculty, Pasundan University, Jl. Dr. Setiabudi No. 9, Bandung, 40153. Indonesia.

Email trisnamegawati299@gmail.com

***Abstract***

*The purpose of this research was to obtained the enhancement of lactic acid levels on salt concentration and the duration in pikel radish fermentation manufacturing. The material which was used in this research was white radish.*

*The basic material which was used in this research was white radish. The research method consisted of two phases, those were the preliminary study and the main study. The preminary study was carried out the analysis of basic material toward the lactid acid levels, water content and total sugar content in radish. The main study was done after the priminary study. It was manufactured of pikel radish by using fermentation. After fermentation, it was carried out the analyisis of lactic acid levels by titration method and analyzes the total of bacteria in pikel radish by TPC method.*

*The result of firts phase research, which was the preliminary study, was obtained that the basic radish materials contained water content component amounted to 94,74%, 0,072% lactic acid levels, and 1,6% the total of sugar content. For the result of the second phase research, was obtained that the salt concentration could affect lactic acid rate. It was Where the radish is fermented with 2,5% salt concentration obtaining the highest lactic acid level, that was research 0,546% with 3,19 pH. Salt concentrations affect the rate of lactic acid, color and texture pikel during fermentation, the higher the salt concentration of the levels of lactic acid produced the lower, the more soft pikel texture and color to yellow. In this study showed that the rate of lactic acid only increased lactic acid levels up to the 12th day and began to decrease lactic acid from the 13th day until 18th day. The total of bacteria during fermentation increased until the 18th day and the total bacteria obtained at the highest salt concentration of 2.5% that is 2,35 x 104.*

*Keywords: Radish, fermentation, pickle and the lactic acid.*

1. **Introduction**

Radish (Rhaphanus sativus L.) is a bulbous vegetable that originated from China and Japan (Santika, 2009). Bulbs elliptical and white as well as a major part for consumption, almost all parts of the radish like leaves and flowers can be consumed. Radish has a strong aroma, the sugar content of 1.9 g radishes and a variety of vitamins that are beneficial to the human body, namely vitamins A, B1, B2, C, E, beta-carotnene, fiber (fiber), and omega-3 high (Shanty, 2014).

The water content in the radish is very high, then the classified material horseradish perishable food. The moisture content of rapeseed, which ranges from 85-95%, so it is good for the growth of microorganisms and accelerate the process of metabolism (Moehamed and Husein 1994 in Asgar, A. and D. Musaddad, 2008).

Almost all kinds of vegetables can be used as raw material for making pikel. Because almost all foodstuffs kinds of vegetables contain sugar. In addition to the usual vegetables made in the raw material for making pikel is kind of tubers and fruits such as carrots, sweet potatoes, purple sweet potato, papaya, mango, and bengkoang often used as a raw material for making pikel.

Seeing the characteristics present in the radish is not much different from the types of tubers are often primarily used for the manufacture pikel that carrot, the radish can be used for making pikel. In making this type of radish pikel time used is the type of daikon (radish locally).

Pikel is the result of processing fruits or vegetables with salt and preserved using acids, or with the addition of sugar and spices as condiments (Vaughn, 1982 in Yulianan and Nurdjanah, 2009).

Pikel made with lactic acid fermentation, but it makes it an easy way. Fermentation is often defined as the process of the breakdown of carbohydrates and amino acids anaerobically, ie without the need for oxygen. Compounds that can be broken in the process of fermentation are carbohydrates, while amino acids can only be fermented by several types of bacteria (Fardiaz, 1992).

Fermentation can be divided into two, namely, aerobic and anaerobic fermentation. During the fermentation process takes place, the sugar in the form of glucose changed into ethanol and various other substrates such as glycerol and lactic acid is referred to as fermentation products. The bacteria that play a role in the fermentation process is able to remodel or alter the compounds contained in food items including compounds that are considered harmful. In addition to the salt fermentation process, can produce certain compounds useful as some of the active compounds produced from the fermentation process is lactic acid, acetic acid, alcohols, aldehydes and gas.

Fermentation time affects the total acid and pH on the end result of making pikel, the longer the fermentation time, the lactic acid concentration increased primarily lactic acid so that the pH goes down (Wulan, 2004 in Yulianan and Nurdjanah, 2009).

Foodstuffs dried generally has a low nutritional value compared to fresh food. During drying can also change color, texture, aroma and other, even though such changes may be limited to a minimum by providing pre-treatment of the foodstuffs (Muchtadi, T, and Ayustaningwaro. F, 2010).

Consuming pikel or fermented products other lactic acid has many benefits for the body which is to facilitate the digestive process in the body due to the pikel very many contain probiotic bacteria (good bacteria) such as Lactobacillus plantarum that could expel flatulence and discomfort associated with disorders (digestion ) such as defecation (BAB). In addition pikel also can reduce the accumulation of fat, reducing the risk of high blood pressure, helps reduce diarrhea caused by certain infections, helps relieve constipation, and helps boost the immune keseluhan (Anonymous, 2012).

1. **Materials and Research Methods**

**Materials**

Raw materials used in this study is a radish-old two days after harvesting (purchased from Agrionolgy Antapani), salt krosok (purchased from the market Ciroyom Bandung). Materials for chemical analysis research that solution Luff Schoorl, phenolpthalein indicator, 30% NaOH, concentrated HCL, H2SO4 6 N, KI powder, amylum, alcohol 70%, Na2S2O3 1 N, 9.5 N HCl, 0.1 N NaOH , distilled water, and the media PCA (Plate Count Agar) (from Lab TP UNPAS).

The tools used in the research process, namely a plastic basin, plastic gloves, spoon, plastic cup, jar 250 ml, knives, cutting boards, incubators, tunnel dryer, tray, analytical balance, the stand, burette, a spray bottle, 250 ml erlenmeyer , funnels, glass beaker 200 ml, 100 ml flask, eksikator, oven, and a pipette.

The tools used for chemical analysis, namely mortar pestle, burette, beaker 100 mL, 250 mL Erlenmeyer flask, volumetric pipette 5 ml, 10 ml and pipette volumetric flask, oven, a petri dish, and pumpkin Kjedahl.

Tools used for microbiological analysis are petri dishes, test tubes and incubators.

**Research Methods**

The implementation of the research conducted preliminary research and primary research**.**

**Preliminary Research**

The preliminary research conducted aims to determine the water content by gravimetric method radishes, turnips levels of lactic acid titration method, and the total sugar content by the method luff schoorl radish (Sudarmadji, S., Haryono, B., and Suhardi. 2010).

**Main Research**

The main research is the manufacture pikel radish using fermentation method with various concentrations of salt and different fermentation time. The main research aims to determine the fermentation time and the concentration of salt right on making turnip pikel to increased levels of lactic acid by titration method and a decrease in pH after fermentation by means of a pH meter (Sudarmadji, S., Haryono, B., and Suhardi. 2010).

**The Experimental Design**

The design of treatment in primary research using a simple graphical method that consists of a variation of the salt concentration (2.5%) (5%), and (7.5%) on the fermentation time (6 days) (12 days) (18 days ) then analysis of lactic acid in pikel radish.

**Design Analysis**

The analytical method used is quantitative analysis of the levels of lactic acid and pH pikel horseradish. Data analysis is averaged and set forth in the Table 1.

Table 1. Levels of lactic acid and pH pikel radish

|  |  |  |
| --- | --- | --- |
| Salt Concentration | Lactic acid (%) | Ph |
| Day - 0 (2,5%) |  |  |
| Day - 6 (2,5%) |  |  |
| Day - 12 (2,5%) |  |  |
| Day - 18 (2,5%) |  |  |
| Day - 0 (5%) |  |  |
| Day - 6 (5%) |  |  |
| Day - 12 (5%) |  |  |
| Day - 18 (5%) |  |  |
| Day - 0 (7,5%) |  |  |
| Day - 6 (7,5%) |  |  |
| Day - 12 (7,5%) |  |  |
| Day - 18 (7,5%) |  |  |

**Response**

Responses conducted in this study are:

**Microbiology Response**. Analyzing the response of microbiological research pikel manufacture of fermented radishes are testing total anaerobic bacteria using the TPC (Total Plate Count) (Fardiaz, 1992).

**Chemical Response**. Chemical response analysis conducted in the research manufacture pikel radishes from the anaerobic fermentation is lactic acid levels analysis by acid-base titration method, the analysis of pH by using a pH meter and analysis of water content in the dried radish pikel by gravimetric methods (Sudarmadji, S., Haryono, B., and Suhardi, 2010).

**3.** **Result and Discussion**

**Preliminary Research**

Table 2. Analysis of water content, lactic acid and total sugar content in radish.

|  |  |
| --- | --- |
| **Component** | **Amount (%)** |
| Water  | 94,74 |
| Lactic acid | 0,072 |
| Sugar  | Glucose /sucrose  | 0,48 |
| Total  | 1,2 |

Based on the results table radishes raw materials analysis results obtained water levels as much as 94.74%, while according to (the Nutrition Directorate of the MOH, 1979 Brilliant Venus Nur Ali and Estu Rahayu, 1995) radish water content was 94.10%. Although there is a difference between the water content of rapeseed is analyzed with horseradish water content according to the Nutrition Directorate , but data analysis results of water content still present in the range of water content according to the Nutrition Directorate.

This is presumably because it is influenced by internal and external factors of the radish itself. As stated by (Pratiwi, T.K. 2011), that the composition of the plant depending on the plant species, and the age structure of the tissue. Various internal and external factors can affect the results in the post-harvest horticulture. Added by (Raharjo, Sri. 2009) that the measure affects respiration of fruits and vegetables, The greater the volume of fruit, the smaller the surface area of ​​the fruit of the unity of weight and vice versa, the smaller the fruit, the greater the surface area of ​​the fruit. Fruit that has a large surface area, then the fruit will have the chance of contact with air (oxygen) were great (oxygen diffuses large), so that the respiration rate is large.

Lactic acid levels obtained in the analysis of the raw material is 0.072%. The function of lactic acid in the raw material horseradish is to compare the results of analysis before the lactic acid fermentation and after fermentation. According to (Zanuck. 2014) in research states that all vegetables are used as raw materials pembutan pikel have nutrients for microbial growth and naturally contains lactic acid, resulting in the manufacture of pikel not need to be added or yeast inoculum. Added by (Phan and Hasu, 1973, in Er. B. Pantastico, 1997) which states that the maximum content of organic acids in vegetable achieved somewhat later than the achievement of maximum carotene, which is then followed by a decline. like the carrots are not tertitrasi high acidity, which showed that the compound buffered cells (buffered) with the real. Some of the acids of the Krebs cycle (oxalate, pyruvate, and isositrat) buried during growth, which means that there is little obstacle on respiration. Phan and Hasu assume that underground, O2 is not so easily obtained as in the air.

Based on analysis of total sugar content in rapeseed derived sugars sucrose or glucose is 0.48% and the total sugar content sebayak 1.2%. According to (USDA Nutrient Database, 2014) that the sugar content in radish 1.86%. Differences component content in radish can be caused by several factors, including different types of horseradish is used, and the age difference radish used for the analysis.

This is consistent with the statement (Er. B. Pantastico, 1997) at an early stage of growth of fruit and vegetables a total sugar content includes reducing and non-reducing sugar is very low. With the increasing ripening, total sugar content rose rapidly at the onset of glucose and fructose, the sudden increase in sugar can be used as a chemical user has the maturity. As mentioned by (Goris, 1969 in Er. B. Pantastico, 1997) which states that the sugar content in carrot growing rapidly approximately 3 months after planting and did not change after harvest. The content of reducing sugars, glucose and fructose was also unchanged, while the ratio between non-reducing sugars and reducing increases exponentially. Therefore, practical changes in sugar content stops long before harvesting, then it can not be used as a chemical clue to maturity.

**Main Research**

**Result Analysis Lactic acid**

A. Lactic acid levels (%) pikel radish

Primary research conducted aims to determine the fermentation time and the best salt concentration with various concentrations of salt are used 2.5%, 5%, and 7.5% to high levels of lactic acid produced can be seen in Figure 3.

**Result Chemistry Analysis**

**4. Conclusion**

.

**5.Refrence**

Figure 1. Increased levels of lactic acid (%) during the fermentation process

Based on the results of a major study of lactic acid levels occur until the 12th day. The highest levels of lactic acid are shown in a salt concentration of 2.5% with the results of 0.546% lactic acid levels at day 12. In other respects, in this study decreased levels of lactic acid from day 13 through day 18 in each concentration. This is not consistent with the statement (Zansuck. 2008) which states that the longer the fermentation time, the number of lactic acid bacteria will continue to rise, followed by elevated levels of lactic acid. But according to (Djunjung and Ansory, 1992) mentions that the lactic acid content will decrease when fermentation takes place faster or less than 14 days. And the characteristics of the species of lactic acid bacteria varied, especially in terms of tolerance to salt, acid and tempratur growth. Differences in these characteristics should be considered in any fermented vegetable product. Particularly when fermenting with dry salting. Can be seen in the image below that the rate of growth of microbes can affect the levels of lactic acid in any concentration.

Figure 2. The rate of growth of microbes on levels of lactic acid salt concentration of 2.5%, 5% and 7.5%

Lactic acid is formed and reached a peak at day 12 which at this stage the growth of lactic acid bacteria are in the stage of accelerated growth, so that the lactic acid bacteria has been through a phase of adaptation to the environment. And decreased levels of lactic acid that occurs on the 18th day which shows that the growth of lactic acid bacteria are under accelerating death phase which leads to reduced substrate thus affecting the metabolic system of lactic acid bacteria. As mentioned by (V. K, Joshi and Somesh, Sharma (2008)) in research, that the increased levels of lactic acid in the salt concentration of 2.5% until the 16th day of 0.6%, and decreased levels of acid lactic between day 16 and day 18 to lactic acid levels up to 0.5%.

Data on average the results of lactic acid in this chard pikel study the lactic acid produced is not too high when compared with the levels of lactic acid produced another pikel products like lettuce and carrots ranged from 0.8 to 1.5% (expressed as lactic acid ) (Tjahjadi. 2011). This is because the total sugar content in rapeseed lower at 1.6% when compared with a total sugar content of carrots from 9.30% and 4.00% so that the fermented cabbage has been slow. This is consistent with the statement (Buckle, 1985 in Nataliningsih. 2009) which states that the sugar found in food ingredients form of glucose will be converted by microbes into lactic acid. Low sugar content of the material resulting in the fermentation process is slow. Added by (Djunjung and Ansory, 1992) that the acidity of 2.0% up to 2.5% of lactic acid will be produced when the sugar contained in considerable amounts in fermented vegetables by means of dry salting.

B. The degree of acidity (pH) pikel radish

Figure 3. The degree of acidity (pH) pikel radish on fermentation time.

Based on the results of this study show that there is a relationship between the length of fermentation to a decrease in pH. Decrease in pH occurred in the fermentation day 0 to day 12 at each concentration. Initial fermentation pH 6 because lactic acid is not yet formed. PH decrease occurs because of the activity of lactic acid bacteria that transform glucose into lactic acid in an anaerobic fermentation process and the longer the fermentation, the lactic acid produced will be increased so that the cause pH pikel getting down. The results of the analysis of pH pikel radishes at each concentration decreased from day 0 to day 12 followed by increased levels of lactic acid, and pH values ​​obtained at the lowest salt concentration of 2.5%. In other respects, in this study pikel pH value has increased or decreased concentration from day 13 to day 18 followed by a decrease in lactic acid levels.

This is consistent with the statement (Munajim, 1988 in Nataliningsih, 2009) that during the process of fermentation, the sugar concentration in the materials will drop, the sugar will turn into lactic acid which was followed by a decrease in pH. This event is accompanied by a chemical change during the process of fermentation, the fermentation process was marked by a decrease in pH. Added by (D. Djungjung and R. Ansory, 1992) that the pH value in the range between 3.3 to 3.5 pikel pikel when stored under conditions approaching anaerobis. Jikalu not so, if there are oxidative yeasts, the pH value will be higher due to loss as a result of acid consumed by bacteria.

C. Effect of pH on levels of lactic acid (%) at a concentration of 2.5%, 5% and 7.5%

Figure 4. Effect of pH on levels of lactic acid (%) at a concentration of 2.5%

Figure 5. Effect of pH on levels of lactic acid (%) at a concentration of 5%.

Figure 6. Effect of pH on levels of lactic acid (%) at a concentration of 7.5%

Based on the three charts above pH analysis results pikel turnip at any salt concentration shows the influence of lactic acid levels to decrease in pH. This is consistent with the statement (Buckle et al. 2007) lactic acid fermentation occurs because of the activity of lactic acid bacteria which convert glucose into lactic acid. During the fermentation process takes place, which is characterized by the onset of gas, the amount of lactic acid increase followed by a decrease in pH. Also added by (Umam, et al., 2012), which states that the decrease in pH is influenced by the content of lactic acid produced by LAB (Lactic Acid Bacteria). Solving sugar in BAL cells (Lactic Acid Bacteria) will produce energy for the activity of probiotic bacteria to produce lactic acid. The formation of lactic acid will lower the pH value and produces a sour taste in the resulting product. Decrease in pH causes the taste to become acidic due to the formation of lactic acid as the main products the metabolism of lactic acid bacteria (Winarno, 1997). The pH value is closely linked to the resulting acid levels.

**Microbiological Response**

|  |  |  |  |
| --- | --- | --- | --- |
| Fermentation Time (Day) | Moisture Concentration 2,5% | Moisture Concentration5% | Moisture Concentration7,5% |
| 0 | 61,83 | 61,41 | 59,80 |
| 6 | 79,34 | 64,35 | 55,79 |
| 12 | 71,02 | 53,57 | 52,44 |
| 18 | 78,96 | 68,41 | 62,54 |

Figure 7.Total microbial growth in pikel radish during the fermentation process

Based on the results of this study show 70,87oC RH, 77.23 ° C, 74.87 ° C; and 74.80 ° C at 0, 6, 12, and 18 days the amount of bacteria during the fermentation at 0, 6, 12, and 18 days with the salt concentration of 2.5%, expressed in units of CFU / g has risen 1.45 x 103, 1.79 x 103, 1.97 x 104 and 2.35 x 104. At a salt concentration of 5% increase in the number of microbes that is 1.37 x 103, 1.75 x 103, 2.45 x 103, and 2.88 x 103. at a salt concentration of 7.5% ie 1.28 x 103, 1.72 x 103, 2.11 x 103 and 2.46 x 103. this is consistent with the statement (Saripah. 1983) mentioning that in 18-24 hours the fermentation process takes place, the salt diffuse into the tissue of vegetables and vegetable nutrients to diffuse out so that the nutrients can be used for the growth of lactic acid bacteria. The longer the fermentation time, the number of bacteria increased. Increasing the number of bacteria during the fermentation caused by the condition of the substrate is still possible for the process of bacterial metabolism. However, bacterial activity decreases due hampered by the acidity generated.

Beginning of fermentation, the number of bacteria increased rapidly because nutrients are available in large quantities. The availability of nutrients in a solution of salt due to the osmotic pressure of the salt to the material so as sugars, vitamins, and minerals will be out of the material. Nutrients are used by the bacteria to grow.

This study also shows the relationship between the number of bacteria in salt concentration. Total microorganisms at concentrations of 2.5%, 5% and 7.5% decline due to the activity of microorganisms can be inhibited by high salt concentrations. This is consistent with the statement (Astuti, 2006), which states that the salt concentration is too high will inhibit the growth of bacteria.

**Chemical response**

Table 3. Results of the analysis of the average water content after drying radish pikel

Figure 8. The moisture content of dried radish pikel.

Based on the analysis of water content data obtained average lowest water content in the salt concentration of 7.5%, when compared to the water content pikel radishes in salt concentration of 5% and 2.5%. The lowest water levels obtained in 7.5% salt concentration in the range of 59.80% -62.54%, when compared to the water content pikel radishes in the market which is 48.36% water content pikel horseradish results of this analysis are still fairly high. This is presumably due to internal and external factors on the process pengringan, long drying method of drying time, and the concentration of salt used. A case mentioned (Wirakartakusumah., A. et al. 1992) that the water content of the material is influenced by several internal and external factors.

Table 4. Results of the analysis of the average levels of lactic acid rapeseed pikel after drying.

|  |  |  |  |
| --- | --- | --- | --- |
| Fermentation time (Day) | Concentration 2,5% | Concentration 5% | concentration 7,5% |
| Lactic acid (%) | Lactic acid (%) | Lactic acid (%) |
| 0 | 1,05 | 0,594 | 0,234 |
| 6 | 0,57 | 0,48 | 0,408 |
| 12 | 0,594 | 0,54 | 0,324 |
| 18 | 0,786 | 0,504 | 0,426 |

Figure 9. Comparison of lactic acid before drying and after drying.

Based on the analysis of data on average levels of lactic acid after drying pikel radish increased. This is not in accordance with the results of the analysis of the levels of lactic acid in horseradish pikel products currently on the market is equal to 0.054%. This is presumably due to the influence of moisture, salinity and drying methods used are different. Drying pikel radish on the research done by cooling and heating. Drying way cool performed at 15oC temperature in the refrigerator for 1 week. Drying how heat is conducted at 70 ° C in the tunnel drayer for 12 hours. Allegedly at that temperature does not vaporize the lactic acid in pikel but simply evaporate most of the water contained therein.

This is consistent with the statement Fardiaz S., (1992), which states that the gram-positive bacteria are generally more resistant to heat dibandingakan with gram-negative bacteria. In addition to heat resistance differs among species of microorganisms heat resistance is also influenced by various parameters contained in microorganisms and environmental parameters. For example, bacteria of the same amount if heated in a physiological salt solution and in nutrient broth will not experience thermal destruction at the same speed (S., Fardiaz. 1992).

Pikel color after dried radish has been changed into a brownish color, this is because the Maillard reaction which is non enzymatic browning reactions which occur due to the reaction between reducing sugars with free amine groups of amino acids or protein. This reaction occurs in many food products are usually consumed daily. Maillard reaction in food can serve to produce flavor and aroma, can cause loss of amino acid availability, loss of nutritional value, antinutrisi formation, formation of toxic components and mutagenic components. Factors - factors that influence the Maillard reaction, which is a type of sugar, acidity (pH), as well as the use of natriurn rnetabisulfit as anti-browning agent to inhibit the Maillard reaction. Maillard reactions are influenced by the type of sugar. On glucose, the longer the sample is heated, the higher its absorbance dun brown color deepened, while sucrose is no change in absorbance significant. This is because glucose is a reducing sugar. The higher the pH, the Maillard reaction will intensify; for optimum Maillard reaction that occurs under alkaline conditions.

**4. Conclusion**

Based on research results pikel radish using a salt concentration of 2.5%, 5%, and 7.5% of the fermentation time 6, 12 and 18 days can be concluded:

1. Radish as a raw material moisture content as much as 94.74%, 0.072% lactic acid levels, and a total sugar content of 1.6%.

2. The salt concentration of 2.5% during the fermentation affect the rate of lactic acid. A salt concentration of 2.5% produces lactic acid levels 0.546% with a pH of 3.19. A salt concentration of 5% produces lactic acid levels of 0.366 with a pH of 3.37. Salt concentration of 7.5% produces lactic acid levels 0.318% with a pH of 4.11.

3. Duration of fermentation affect the rate of formation of lactic acid. Increased levels of lactic acid occurs until the 12th day and decreased from day 13 to day 18. Samples with salt concentration of 2.5% produces lactic acid levels of 0.546% and on day 18 amounted to 0.234%. Samples with a salt concentration of 5% produces lactic acid levels on day 12 amounted to 0.366% on the day and the 18th day of 0.174%. Samples with salt concentration of 7.5% produces lactic acid levels on day 12 amounted to 0.316% and on day 18 amounted to 0.162%.

4. Total bacterial samples obtained at the highest salt concentration of 2.5% fermented for 18 days, 2.35 x 104.

5. The lowest water content after the drying is generated by sample with 7.5% salt concentration is 0.234%.

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