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The Effect Of Sugar Dose And Fermentation Time On The Quality Of Sauerkreut As A Source Of Probiotic

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Abstract. This study aims to determine the effect of sugar dosage and fermentation time on the quality of sauerkraut as a source of probiotics. This study used an experimental method in a 3x3 factorial completely randomized design (CRD). The first factor is three treatments of sugar concentration (A), namely A1 (2%), A2 (3.5%), and A3 (5%). The second factor is three treatments of fermentation time (B), namely B1 (5 days), B2 (7 days), and B3 (9 days). The results showed that there was no interaction ($P>0.05$) between factor A and factor B in the water content, but the water content had a significant effect ($P<0.05$) separately in factor A and factor B. In total LAB, there was a very significant interaction ($P<0.01$) between factor A and factor B, and in factor A and factor B. In pH, there was a significant interaction ($P<0.05$) between factor A and factor B, but a very significant effect ($P<0.01$) in factor A and factor B. In acid content there was a significant interaction ($P<0.05$) between factor A and Factor B, but a very significant effect ($P<0.01$) on factor A and factor B. This study concludes that cabbage processed into sauerkraut can be used as a probiotic supplement. The addition of 3.5% sugar and 7-day fermentation resulted in 91.11% water content, 3.43 pH, 2.93% total acid, and 7.4×10^9 total LAB.

Keywords: Sauerkraut, Sugar concentration, Fermentation

Abstrak. Penelitian ini bertujuan untuk mengetahui pengaruh dosis gula dan lama fermentasi terhadap kualitas sauerkraut sebagai sumber probiotik. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) pola factorial. Faktor pertama adalah konsentrasi gula (A) dengan 3 perlakuan yaitu Perlakuan A1 adalah dosis konsentrasi gula 2%, perlakuan A2 adalah dosis konsentrasi gula 3,5% dan perlakuan A3 adalah dosis konsentrasi gula 5%. Faktor kedua adalah lama fermentasi (B) dengan 3 perlakuan yaitu perlakuan B1 adalah 5 hari, perlakuan B2 adalah 7 hari dan perlakuan B3 adalah 9 hari. Peubah yang diamati adalah pengukuran kadar air, total bakteri asam laktat, pH dan total asam. Hasil penelitian menunjukkan bahwa pada kadar air tidak terdapat interaksi ($P>0,05$) antara faktor A dan faktor B, akan tetapi kadar air pada faktor A berpengaruh nyata ($P<0.05$) dan faktor B juga berpengaruh nyata ($P<0.05$). Total BAL terdapat interaksi berpengaruh sangat nyata ($P<0,01$) antara faktor A dan faktor B, akan tetapi total BAL pada faktor A berpengaruh sangat nyata ($P<0.01$) dan faktor B berpengaruh sangat nyata ($P<0.01$). pH terdapat interaksi berpengaruh nyata ($P<0,05$) antara faktor A dan Faktor B, akan tetapi pH pada faktor A berpengaruh sangat nyata ($P<0.01$) dan faktor B berpengaruh sangat nyata ($P<0.01$). Kadar asam terdapat interaksi berpengaruh nyata ($P<0,05$) antara faktor A dan Faktor B, tetapi kadar asam faktor berpengaruh sangat nyata ($P<0.01$) dan faktor B berpengaruh sangat nyata ($P<0.01$). Kesimpulan dari penelitian ini adalah kol yang diolah menjadi sauerkraut dapat digunakan sebagai supplement probitik. Penambahan gula 3,5 % dan lama fermentasi 7 hari memiliki kualitas kadar air pada kisaran (91,11%) pH (3,43), total asam (2,93%) dan total BAL ($7,4 \times 10^9$).

Kata kunci: Sauerkraut, Konsentrasi gula, Fermentasi

Introduction

The banned antibiotics Growth Promotor (AGP) in livestock by the government has raised concerns among breeders and in livestock industry. AGP is a growth booster to increase livestock productivity, but it carries a potential risk in the form of antibiotic residues left in the treated product which can harm consumers. Given this danger, researchers are trying to replace antibiotics with natural ingredients, including probiotics.

According to Sopandi *et al.* (2014), probiotics are products that contain living microorganisms that can help directly increase body resistance to intestinal pathogens and prevent disease. Probiotics can be made by fermenting plant materials or vegetables such as cabbage. Cabbage has an important for human health because it contains vitamins and minerals the body needs, so the demand for this vegetable continues to increase. Swains *et al* (2014) added that cabbage contains any nutritional components that include 5.8% carbohydrates, 3.2% sugar, 1.28% protein, 0.1% fat and 2.5 grams of fiber. Accordingly, cabbage can be used as a medium to grow microorganisms such as lactic acid bacteria for fermentation process. Cabbage is easily wilted, damaged, and rotten, so fermentation can extend its shelf life and give it a distinctive taste as a fermented product known as sauerkraut.

Sauerkraut fermentation use 2-2.5% salt or NaCl (Susilowati *et al.,* 2016) and sugar (Cynthia *et al*., 2020). High sugar content in raw materials is very decisive in the formation of acid levels, especially lactic acid in the sauerkraut manufacturing process. The addition of 2% sugar concentration in making sauerkraut can maximize the fermentation process (Zubaidah *et al*., 2020). Fermentation duration also affects sauerkraut manufacture because, as reported by Chintya *et al* (2020), sugar concentration is directly proportional to fermentation duration, in which the higher the sugar concentration, the faster the fermentation. This is due to the fact that high sugar concentrations are fuels to microbial growth. Chintya *et al* (2020) reported that fermenting sauerkraut requires fermentation for about 48-72 hours.

Scientific research on sauerkraut fermentation from cabbage with the addition of sugar and fermentation time has been scarcely published. Therefore, further research is crucial to determine the best effect of adding sugar and fermentation time in the treatment.

Materials and Methods

This research was carried out at the research laboratory of the Faculty of Agriculture, Animal Husbandry Study Program, University of North Sumatra from February to May 2022. The raw materials used in this study were cabbage, non-iodized table salt, sugar, NaOH, alcohol, distilled water, and PP indicator. The research equipment was glassware (test tubes, petri dishes, Erlenmeyer, dropper pipettes, stirrers, measuring cups, and jars for fermentation), digital scales, pH meters, autoclaves, airflow laminators, incubators, vortex mixers, micro pipettes, hotplates, Bunsen lamps, micropipettes, and tips.

Sauerkraut was made by wilting the cabbage for one night, then removing the outer cabbage leaves, damaged parts, and the heart. Then, the cabbage was washed thoroughly, thinly sliced (± 2-3mm), mixed with salt (2.5% of the weight of the cabbage and incorporated with sugar according to treatment, stirred until smooth then put into the jar with a little pressure to make it solid. Then, the jar was covered with a plastic to ensure airtight, and the cabbage was let fermented according to the treatment at room temperature, then stored in the refrigerator.

The test for determining the pH value was carried out by the method of Apriyantono, *et al* (1989) using a pH meter that had been calibrated with a buffer solution at pH 4 and 7. The water content test was carried out using the AOAC method (1995). The total microbial determination test was carried out using the Fardiaz method (1992).

The variable was calculated using the formula:

$$Moisture\ content\ (\%) = \frac{Weight\ loss\ after\ drying\ (g)}{Sample\ weight\ (g)} x 100\%$$

$$Lactic\ acid\ (\%) = \frac{NaOH\ Volume\ x\ N\ x\ 900/1000}{Sample\ volume} x 100\%$$

Where:

N= Normality of the NaOH solution used as titer

$$Total\ colonies\ \left(\frac{CFU}{g}\right) = \frac{Number\ of\ colonies\ calculated}{Diluting\ factor}$$

Table 1. Water content (%) of sauerkraut with different sugar concentrations and fermentation times

Factor A (Sugar Concentration)	Factor B (Fermentation Time) B1 (5)	B2 (7)	B3 (9)	Average
A1 (2%)	91.39	91.10	90.82	91.10
A2 (3.5%)	91.50	91.11	91.01	91.01
A3 (5%)	91.72	91.24	91.08	91.08
Average	91.53[a]	91.15[a]	90.97[a]	

Note: The same letter notation indicates that the treatment has no significant effect (P>0.05)

Observational data were analyzed for variance using a Factorial Completely Randomized Design and any significant effect was subjected to DMRT based on Steel and Torrie (1995) with a 3x3x3 factors. This research applied factors. The first factor was three treatments of sugar concentration (A), namely A1 (2%), A2 (3.5%), and A3 (5%), and the second factor was treatments of fermentation time (B), namely B1 (5 days), B2 (7 days), and B3 (9 days).

Results and Discussion

Effect of Treatment on Sauerkraut Moisture Content

The water content of sauerkraut with different doses of sugar and fermentation time (in days) are presented in Table 1. The analysis of variance showed that incorporating sugar concentration in the sauerkraut making and applying different fermentation times have significantly (P <0.05) affected sauerkraut water content.

The highest average water content was 91.10 (2%) observed in treatment A2 of while the lowest was 91.01 (3.5%) also in A2. The water content across treatment was similar. The result of microbial activity here was the degradation of sugar into carbon dioxide and water, and thus sugar determines the development of bacteria in fermentation process. It is in accordance with Putu *et al* (2020) that during the fermentation process, lactic acid bacteria break down sugar through metabolic system to produce energy for growth, and thus, in addition to nutrients form fermented ingredients, sugar is a food source for bacteria to develop. Regarding the fermentation time, the highest average water content was 91.53 observed in treatment B1 (5 days) while the lowest was 90.97 in B3 (9 days). The fermentation time (in days) can reduce water content. Abdillah *et al* (2014) claimed that the longer the fermentation, the less the oxygen content because the oxygen is assumed to have been used by the bacteria for respiration and for producing energy, carbon dioxide and water. In this study, the duration of fermentation in the DMRT test was not significantly different but decreasing in each treatment.

The absence of effect of interaction between sugar concentration and fermentation duration on water content (P>0.05) is because the dose of sugar does not contribute to determining water content, and use of the same materials for all treatments in this study may be perceived as producing the same level of water content. Microbial growth resembles a competition because the same nutrition is needed by many organisms within one area (Abdillah *et al.,* 2014)

The water content obtained in this study ranged from 90.82% to 92.72%. This figure is similar to Nakdiyani *et al* (2019) who researched water content of sauerkraut in cabbage and carrots using different salt concentrations and yielded 91.11%.

Effect of Treatment on Total LAB

Total LAB from sauerkraut made of different doses of sugar and duration of fermentation (in days) are presented in Table 2. The results of analysis of variance showed that adding sugar concentration has highly significantly affected (P < 0.01) and applying different fermentation time also highly significantly affected (P < 0.01) the total LAB of sauerkraut

Table 2. Total lactic acid bacteria (CFU/ml) from sauerkraut with different sugar concentrations and fermentation time in 10^9

Factor A (Sugar Concentration)	Factor B (Fermentation Time)			Average
	B1 (5)	B2 (7)	B3 (9)	
A1 (2%)	4.1^e	5.6^d	5.7^d	5.1
A2 (3.5%)	6.3^c	7.4^a	7.2^a	6.9
A3 (5%)	6.7^b	7.4^a	7.3^a	7.1
Average	5.7	6.8	6.7	

Note: Different letter notations indicate significantly different effects (P<0.01)

The highest average total bacteria was 7.1 observed in treatment A3 (5%) while the lowest was 5.1 in treatment A1 (2%). This result is due to the concentration of sugar is a source of energy for microbes in developing. It is in accordance with Koesoemawardani et al. (2013) that the addition of sugar into the medium for bacteria to grow will be utilized by LAB as a carbon source for their performance, and therefore, the longer the fermentation, the less the sugar. Incorporating sugar with different concentrations into sauerkraut making results in different rate of LAB growth, and therefore, sugar is closely related to LAB development.

The highest average total bacteria was 6.8 treatment B2 (7 days) while the lowest was 5.7 in B1 (5 days). Different fermentation time across treatments has increased LAB growth. It is in accordance with Yunus *et al* (2015) that the longer incubation time of LAB will allow the LAB to ferment sugar to produce lactic acid and energy for growth. Meanwhile, the interaction between the addition of sugar concentration and fermentation time showed a very significant effect (P<0.01) on the total lactic acid bacteria of sauerkraut. This indicates that the high total lactic acid bacteria in the treatment is due to the sugar dose and the fermentation days are interdependent. According to Kechagia (2013), although information about the minimum effective concentration is still insufficient, it has been known that probiotic products must have a minimum concentration of 10^6 CFU/mL or gram. Accordingly, the sauerkraut product in this study contained a higher concentration.

Effect of Treatment on *Sauerkreut* pH

The degree of acidity (pH) of *sauerkraut* with different doses of sugar and duration of fermentation (in days) are presented in Table 3.

The results of the analysis of variance showed that adding sugar concentration and employing different fermentation time showed a very significant effect ($P < 0.01$) on the degree of acidity (pH) of sauerkraut. The highest average degree of acidity (pH) was 3.93 in treatment A1 (2%) while the lowest was 3.58 in treatment A3 (5%). According to Adawiyah (2014), lactic acid bacteria (LAB) is a group of bacteria that produce lactic acid as the main product in carbohydrate metabolism. Lactic acid produced in this way will reduce the pH value of the growth environment and generate a sour taste and vice versa if the total LAB is low, so the pH value will be high.

Table 3. Degree of acidity (pH) of sauerkraut with different sugar concentrations and fermentation times

Factor A (Sugar Concentration)	Factor B (Fermentation Time)			Average
	B1 (5)	B2 (7)	B3 (9)	
A1 (2%)	4.53^a	3.77^b	3.50^c	3.93
A2 (3.5%)	3.87^b	3.43^c	3.47^c	3.59
A3 (5%)	3.80^b	3.47^c	3.47^c	3.58
Average	4.07	3.56	3.48	

Note: Different letter notations indicate significantly different effects (P<0.05).

Table 4. Acid content (%) of sauerkraut with different sugar concentrations and fermentation times

Factor A (Sugar Concentration)	Factor B (Fermentation Time)			Average
	B1 (5)	B2 (7)	B3 (9)	
A1 (2%)	1,82^{b}	2,23^{b}	2,27^{b}	2,11
A2 (3,5%)	1,91^{b}	2,93^{a}	2,73^{a}	2,53
A3 (5%)	1,99^{b}	2,71^{a}	3,00^{a}	2,57
Average	1,91	2,63	2,67	

Note: Different letter notations indicate significantly different effects (P<0.05)

The highest average degree of acidity (pH) was 4.07 in treatment B1 while the lowest was 3.48 in treatment B3. Low pH in each treatment is due to the accumulation of lactic acid from LAB metabolism during the fermentation process, so that the pH value will tend to decrease as the length of fermentation increases. Nurjannah (2017) report decreased pH value indicates the formation of organic acids due to microbial metabolism. A lower acidity degree also increases the acid concentration in the sauerkraut fermentation liquid.

While the interaction between the addition of sugar concentration and the length of fermentation showed a significant effect (P<0.05) pH of sauerkraut. This indicates that the pH in the treatment is due to the interdependent relationship between the sugar dose and fermentation duration.

The pH of sauerkraut made with different doses of sugar and fermentation durations ranged from 3.43 to 4.53. This figure is lower than that reported by Nakdiyani et al (2019) which reported in 3.70- 4.80.

Effect of Treatment on Acid Levels

The acid content of sauerkraut with different doses of sugar and duration of fermentation can be seen in Table 4.

Results of the analysis of variance showed that adding sugar concentration and applying different fermentation time showed a very significant effect (P < 0.01) on acid content of sauerkraut. The highest average acid content was 2.57 in treatment A3 (5%) while the lowest was 2.11 in A1 (2%). According to Edam (2018) reported that the most important characteristic of lactic acid bacteria is the ability to ferment sugar into lactic acid so that acid levels increase.

The highest average acid content in the fermentation duration treatment was 2.67 in treatment B3 (9 days) while the lowest was 1.91 in B1 (5 days). It is in line with Yunus et al. (2015) that the longer the fermentation, the higher the total bacteria and total acid produced due to a lot of time is available for lactic acid bacteria to remodel the nutrients contained in the substrate, thus allowing the accumulation of organic acids such as lactic acid in a greater quantity.

The interaction between the addition of sugar and the length of fermentation showed a significant effect (P<0.05) on the acid content of sauerkraut. This is due to the fermentation duration and the high total content of lactic acid bacteria.

Sauerkraut made of different doses of sugar and fermentation duration produced acid levels ranging from 1.82% to 3.00%. This figure is similar to 0.8-2.6% on fermented cabbage with different concentrations of NaCl and fermentation time (Edam, 2018), but higher than 0.95% on sauerkraut made from cabbage with variations in salt concentration (Hayati et al. 2017).

CONCLUSION

Cabbage processed into sauerkraut is a feasible probiotic supplement. Applying 3.5% sugar and 7-day fermentation time produced sauerkraut with 91.11% water content, 3.43 pH, 2.93% total acid, and 7.4 x 109 total LAB.

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