

The Duracy of Fermentation Effect on the Chemical Quality of Kimchi Krai (*Cucumis sativus L.*) as Probiotics

Halimatun Nafisah*, Rudiana Agustini

Chemistry Department, Universitas Negeri Surabaya, Surabaya, Indonesia

*E-mail: halimatus.19059@mhs.unesa.ac.id

Received: January 4, 2024. Accepted: January 27, 2024. Published: March 27, 2024

Abstract: This study aims to describe the effect of fermentation duration on the chemical quality of kimchi krai as a food containing probiotics, including total LAB, total titratable acid, pH, antioxidant activity, and level of liking. This research used laboratory experimental methods, and data analysis was done descriptively. Several stages were carried out, including sample making, sample preparation, sample extraction, total LAB test, pH test, total titratable acid test, antioxidant activity test with DPPH method to obtain IC₅₀ value, and organoleptic test. This study used fermentation durations of 8 hours, 16 hours, and 24 hours. The best chemical quality of kimchi krai was obtained in 24-hour fermentation with a total LAB of 6.1×10^7 , pH value decreased from pH 4.75 in 8-hour fermentation to 3.85 in 24-hour fermentation, while for total titratable acid with the highest value of 0.62%, the strongest antioxidant activity with IC₅₀ 429.86 ppm. Based on the results, the very weak category includes antioxidant activity in kimchi krai (*Cucumis Sativus L.*). However, the longer the fermentation process of kimchi krai (*Cucumis Sativus L.*), the greater the ability to inhibit free radicals.

Keywords: Krai; Total LAB; Fermentation; Chemical Quality.

Introduction

Kimchi is one of the foods that are rich in probiotics. This raw vegetable preparation is a fermented product with spicy seasonings [1]. Vegetables rapidly deteriorate and decay if not handled cautiously after being harvested. To prolong their freshness, the creation of kimchi is a viable solution. The process involves immersing the vegetables in a brine for a few hours, rinsing, and applying a seasoning composed of fish sauce, garlic, ginger, and red chili powder. As a result of fermentation, kimchi develops a tangy flavor reminiscent of pickles [2].

Kimchi contains probiotics, namely lactic acid bacteria, that play a role in fermentation and produce high levels of lactic acid. Achieving optimal lactic acid fermentation hinges on meticulously managing variables influencing lactic acid bacteria's proliferation. Typically, during the initial stages of fermentation, the dominant strain of bacteria responsible for growth is *Leuconostoc mesenteroides*. This is what can facilitate the digestive system. Kimchi is also believed to have antioxidant properties [3]. Probiotics are live microorganisms that, if given in adequate amounts, will have a healthful effect on the recipient. Adding probiotic bacteria in food and beverages improves human health by reducing pathogenic bacteria in the gut and stimulating immunity [4].

The krai plant that will be used as the basic ingredient of kimchi in this study is a cucumber-type vegetable plant with a green color with white stripes. This plant has low calories, namely 15 calories per 100 grams, does not contain saturated fat or cholesterol, and can be used as fiber. Its fiber content is about 0.5 grams per 100 grams, with a potassium content of 147 mg, sodium, and essential electrolytes. Krai contains vitamin A, which

functions as an antioxidant and is beneficial for preventing inflammation and infection. In addition, krai contains flavonoid compounds. According to research, flavonoids and phenolics are essential as natural antioxidant ingredients. Compared to unprocessed krai, kimchi has a more excellent fiber content that will increase during fermentation. The average fiber in some types of kimchi is 2 grams per 100 grams [5].

One of the ingredients found in kimchi is antioxidants. Antioxidants are compounds that can absorb or neutralize free radicals to prevent degenerative diseases [6]. The prevention of oxidative stress necessitates the presence of antioxidants. Antioxidants, being highly susceptible to oxidation, are readily oxidized by free radicals. This process safeguards molecules within cells from the detrimental effects of oxidation caused by free radicals or reactive oxygen [7].

Based on this, research was conducted on the chemical quality of kimchi to find new ingredients, such as krai, that are safe as antioxidants and easy to digest for the body, considering that the good bacteria in kimchi can also help reduce inflammation in the digestive system because it is rich in lactic acid bacteria resulting from fermentation. This study is also expected to provide information on the advantages of krai used as kimchi compared to unprocessed krai and the best length of fermentation time for consumption.

Research Methods

Tools and Materials

The tools used for this research are laboratory equipment made of glass such as erlenmeyer, a measuring

How to Cite:

Nafisah, H., & Agustini, R. (2024). The Duracy of Fermentation Effect on the Chemical Quality of Kimchi Krai (*Cucumis sativus L.*) as Probiotics. *Jurnal Pijar Mipa*, 19(2), 308–313. <https://doi.org/10.29303/jpm.v19i2.6415>

cup, a volumetric flask, a test tube, a micropipette, vortex, measuring pipette, incubator, analytical balance, centrifugator, sonicator, UV-Vis 1800 spectrophotometer, vortex, pH meter, Petri dish, and laminar.

While the materials needed include: distilled water, DPPH crystals, ethanol pro analysis (p.a), CaCO₃, NaCl powder, MRSA liquid media, 0.1 N NaOH, pH 7 and pH 4 buffer powder, 70% alcohol, and PP indicator, krai, garlic, onions, fish sauce, salt, chili powder, and sugar.

Research Procedure

Making Krai Kimchi Samples

Making krai kimchi samples begins with salting the krai for approximately 30 minutes. The salt used was 2 percent of the kimchi's weight. Furthermore, making kimchi krai sauce is done by mixing chili powder, garlic, onions, green onions, sugar, and salt as much as 1%. After the salting process is complete, the krai is washed 2-3 times and then stirred together with the sauce that has been made. And divided into three containers for fermentation for 8 hours, 16 hours, and 24 hours.

Total LAB Testing

The total lactic acid bacteria was measured using the entire plate count method by diluting 0.85% NaCl solution and MRSA media dissolved with 1% CaCO₃. The plates were incubated in an inverted position at 37° C. for 48 hours. Then, the microbes were counted with units (CFU/ml).

Total Acid Tertiration Testing

The kimchi sample of as much as 4 mL was diluted in a 100 mL volumetric flask and then entered as much as 25 mL entered into an erlenmeyer flask and added a phenolphthalein indicator as much as 2-3 drops and titrated with 0.1 N NaOH. Titration will be stopped if there is a permanent pink color change. Total titratable acid (TAT) is expressed in percent lactic acid. The formula calculates the total titratable acid:

$$TAT = \frac{V \times N \times P \times BM \times 100\%}{B \times 1000}$$

Description:

V: Volume of NaOH titrated (mL)

N: Normality of NaOH

P: Number of dilutions

BM: Lactic acid molecular weight

B: Sample weight (mg) [8].

pH Testing

The pH measurement was carried out using a pH meter. The pH meter was calibrated first with buffer for pH 4 and pH 7 according to the pH range of kimchi. Measurements were made by dipping the pH meter electrode into 10 ml of sample.

Antioxidant Activity Testing

Antioxidant activity testing was carried out using the DPPH method with sample concentrations of 200, 400, 600,

800, and 1000 ppm and measured absorbance at a maximum wavelength of 518.0 nm.

Results and Discussion

Effect of Fermentation Duration on the Growth of Lactic Acid Bacteria.

This test aims to determine the effect of fermentation duration on the growth of lactic acid bacteria in kimchi krai. The test used de Mann Regose and Sharpe Agar (MRSA) media with additional CaCO₃. The method used in this study is Total Plate Count (TPC) or total plate count (ALT) to see the number of lactic acid bacteria that can grow. The principle of this method is to grow microbial cells still alive in the media. The media acts as a nutrient for the lactic acid bacteria, so the microbial cells will multiply and form colonies that can be seen directly using the naked eye. The reaction that occurs is as follows:

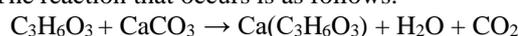


Figure 1. Colonies growing in the clear zone during the incubation process

Lactic acid bacteria can be directly identified because there is a clear zone on the media due to the reaction between lactic acid produced by LAB and CaCO₃ during the incubation period to produce Ca-lactate. The results of lactic acid bacteria growth can be seen in Table 1.

Table 1. Effect of Fermentation Time on the Growth of Lactic Acid Bacteria of Kimchi Krai

No	The Duracy of Fermentation	Total LAB (log CFU/mL)
1	8	6.77 ^a ±0.15
2	16	6.96 ^a ±0.11
3	24	7.76 ^b ±0.14

Notes: Values followed by different letters indicate significant differences (p<0.05).

The statistical analysis results using *One Way Anova* showed a significance value of $p < 0.05$, indicating fermentation duration's effect on the growth of lactic acid bacteria contained in kimchi krai. Furthermore, a Post Hoc Duncan analysis was conducted to identify significant differences in each treatment. The statistical results showed a substantial change in the 16-24 hour fermentation, but there was no significant difference in the 8-16 fermentation time. Based on the following data, it can be stated that the length of fermentation affects the growth of lactic acid bacteria in kimchi krai with optimum growth of LAB at 24 hours of fermentation to 7.76 log CFU/mL.

Salt addition and temperature regulation are important factors during fermentation. Kimchi krai that has been made will be immediately put into the refrigerator to ferment correctly. This is supported by Lestari's (2016)

research, which found that the ideal salt concentration for making radish kimchi is 3%, and the perfect fermentation temperature is 4-10°C. In this study, salt concentration significantly impacted moisture content, total soluble solids, total lactic acid, vitamin C, fiber content, aroma, taste, and texture of radish [9].

In this study, the process used during fermentation is a short-term fermentation process. This is related to salt use, which must be limited to concentrations ranging from 2.5% to 10%. According to Pradani et al. (2009), their research confirms that the concentration of salt solution utilized impacts the fermentation of vegetables. If the salt solution level falls below 2.5%, it promotes the proliferation of spoilage and proteolytic bacteria responsible for protein breakdown. Conversely, excessively high salt concentrations exceeding 10% are not advisable because the use of salt that is too high can inhibit heterofermentative bacteria, especially *Leuconostoc Mesenteroides*, which is a bacterium that grows at the beginning of fermentation [10]. It can stimulate the growth of homofermentative bacteria in excess and produce less carbon dioxide. At the same time, carbon dioxide is needed to release oxygen trapped in vegetable tissue. Because of the remaining oxygen, yeast can grow and contaminate the product [11].

Effect of Fermentation Duration on Total Titratable Acid (TTA)

This test is intended to describe how the length of fermentation affects the increase in total acid formed. Total acid is a product resulting from the metabolism of lactic acid bacteria during fermentation and can be measured by the acid-base titration method. The basic principle of acid-base titration is to neutralize the acid or base, where an easy red color change in the sample will mark the end point of the titration.

Table 2. Effect of Fermentation Duration on the Decrease of TTA Value of Kimchi Krai.

No	The Duracy of Fermentation	TTA (%)
1	8	0.31 ^a ±0.04
2	16	0.48 ^b ±0.07
3	24	0.62 ^b ±0.08

Notes: Values followed by different letters indicate significant differences (p<0.05).

The statistical analysis results using *One Way Anova* showed a significance value of p < 0.05, indicating the effect of fermentation duration on the increase in TTA value. Furthermore, *Post Hoc Duncan* analysis was conducted to identify significant differences in each treatment. Statistical results on 8-16 hours fermentation showed a considerable difference, but there was no significant difference on 16-24 hours fermentation. This indicates a faster increase in TTA content after a specific time limit. It can be concluded that the length of fermentation time increases the TTA value of kimchi krai, where the TTA content rises to 0.62% after 24 hours of fermentation.

Lactic acid produced during fermentation can improve flavor and increase acidity or decrease pH. The formation of lactic acid and metabolites of LAB during the

fermentation process will affect total LAB, pH, acidity, flavor, texture, and liking [12].

The fermentation rate of kimchi is affected by salt concentration and temperature. Kimchi is best consumed with 0.6-0.8% titrated acid (pH 4.2), 3% sodium chloride, and a reasonably high organic acid content. In this case, the research results show that the optimal pH of kimchi reached 4.2 after 16 hours of fermentation, and the maximum pH was 3.85 after 24 hours of fermentation. The TTA value also reached 0.62% after 24 hours of fermentation [13].

Effect of Fermentation Duration on pH Decrease

The pH test was conducted to determine the degree of acidity in the sample, and it was measured using a pH meter. The pH test results are shown in Table 3.

Table 3. Effect of Fermentation Duration on the Decrease of Kimchi Krai pH.

No	The Duracy of Fermentation	pH
1	8	4.75 ^a ±0.02
2	16	4.20 ^b ±0.15
3	24	3.85 ^c ±0.11

Notes: Values followed by different letters indicate significant differences (p<0.05).

A significant difference was found based on the results of the One-way anova statistical test, which aims to indicate the effect of fermentation duration on reducing the pH of krai kimchi. Furthermore, *Duncan's Post Hoc* test was conducted to see the actual differences in each treatment. The results showed a significant difference in the 8-24 hours fermentation time. The length of fermentation affects the decrease in pH value in kimchi; it can be seen in Table 3. that the pH will decrease as the fermentation process progresses and reaches a pH of 3.85 after 24 hours compared to the pH at 8 hours of fermentation, which is 4.75.

The decrease in pH in kimchi occurs with natural fermentation. Kimchi fermentation is initiated by various microorganisms found in the ingredients. Still, kimchi fermentation is gradually dominated by lactic acid bacteria, which play an essential role in the flavor of kimchi and acause This states that the higher the value of total titratable acid, the more the pH value of kimchi will decrease.

Figure 2 proves the correlation between an increase in total LAB, a decrease in pH, and an increase in TAT value.

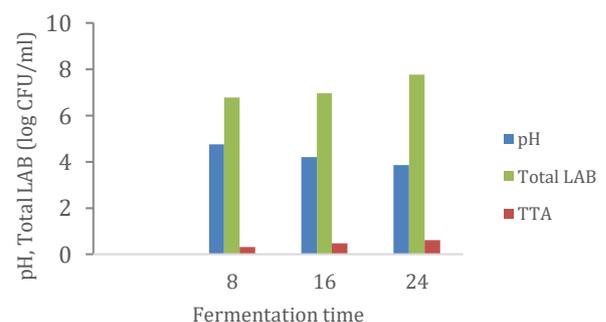


Figure 2. Graph of LAB growth, pH decrease, and TAT increase in kimchi krai based on the length of fermentation time.

Based on the graph above, it is concluded that the longer the fermentation time, the more the value of total lactic acid bacteria, the total titratable acid will increase, and the more the pH value will decrease. The kimchi standard based on Codex CXS 223-2001, updated in 2017, states that kimchi is considered to meet the Codex international percent lactic acid standard, with salt content (NaCl) ranging from 1.0 to 4.0% and total acidity (lactic acid) of no more than 1.0%. Kimchi should be red like red pepper, have a spicy and salty flavor, and be sour. The texture should be chewy, crunchy, and moderately firm [15]. Tayo and Akpeji (2016) stated that probiotic foods should generally have at least 10^6 CFU/g of active and alive organisms when consumed [16].

Effect of Fermentation Duration on Antioxidant Activity

The DPPH method was utilized to analyze antioxidants, relying on the substance's capacity to diminish the free radical known as 1,1-diphenyl-2-picrylhydrazyl (DPPH). The fundamental concept behind the DPPH method involves the existence of hydrogen atoms within antioxidant compounds that bond with unpaired electrons in radical compounds, transforming from free radicals to non-radical compounds. A color change to yellow indicates this transformation. Free radicals are molecules that have excess electrons and can cause damage to body cells by taking electrons from surrounding molecules. Antioxidants work by absorbing free radicals and preventing oxidative damage. The amount of antioxidant activity can be expressed by the IC₅₀ value, which is the concentration of antioxidant compounds that inhibit 50% of free radicals. In this study, the maximum wavelength obtained was 518.0 nm. The results of antioxidant activity testing can be seen in Table 4.

Table 4. Effect of Fermentation Duration on IC₅₀ Value of Kimchi Krai

No	The Duracy of Fermentation (hour)	IC ₅₀ (ppm)
1	8	469.46 ^b ±0.65
2	16	457.68 ^c ±1.74
3	24	429.86 ^d ±0.53

Notes: Values followed by different letters indicate significant differences (p<0.05).

The statistical analysis results using One Way Anova showed a significance value of $p < 0.05$, indicating the effect of fermentation duration on antioxidant activity. Further tests were conducted using *Post Hoc Duncan* analysis to identify differences in each sample treatment. Statistical results showed a significant difference in the 8-24 hours fermentation time. The length of fermentation is known to influence antioxidants characterized by decreasing IC₅₀ values. The IC₅₀ value at the beginning of fermentation was 528.13 ppm, then gradually reduced to 429.86 ppm after fermenting for 24 hours.

Storage temperature, preparation composition, type and thickness of packaging material, humidity, oxygen content, and light exposure are some additional factors that affect the ability of probiotics to produce bioactive compounds, including antioxidants, during storage [17]. This explains why the longer the fermentation, the stronger the antioxidant activity, whereas the greater the number of

probiotics or, in this study using a focus on probiotic lactic acid bacteria, the stronger the antioxidant activity produced. The following is a graph of antioxidant activity characterized by a decrease in IC₅₀.

Based on antioxidant testing, it can be seen that the IC₅₀ value continues to decline, which indicates the increasing antioxidant activity of kimchi krai samples. At 24 hours of fermentation, the smallest IC₅₀ value and the highest antioxidant activity of 429.86 ppm were obtained. With such an IC₅₀ concentration, it can be stated that the antioxidant in kimchi krai is very weak. An antioxidant is classified as highly potent if its IC₅₀ value falls below 50, strong if it ranges from 50 to 100, medium if it falls between 100 and 150, and weak if it falls between 151 and 200. Any compound with an IC₅₀ value exceeding 200 can be categorized as very weak [18].

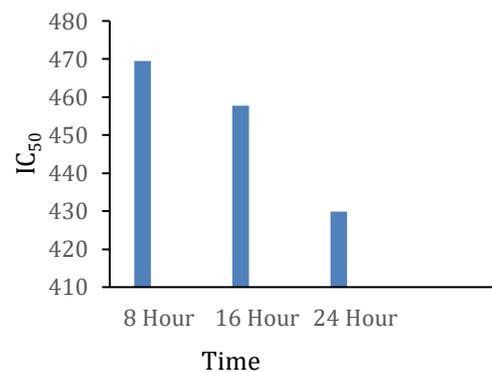


Figure 3. Antioxidant activity graph based on IC₅₀ value reduction

The DPPH method is a simple, quick, and easy method for screening the radical-capturing activity of several compounds; besides that, this method is proven to be accurate, effective, and practical [19]. Flavonoid compounds will capture DPPH free radicals. DPPH free radicals will oxidize flavonoids to produce a more stable radical form, an extreme with low reactivity. Flavonoids donate hydrogen radicals from their aromatic rings to reduce toxic free radicals, resulting in resonance-stabilized flavonoid radicals and making them non-toxic [20]. This is what makes the absorbance of DPPH continue to decrease, and there is a gradual change from dark purple to yellowish. The discoloration occurs when DPPH radicals are captured by antioxidants that release hydrogen atoms to form stable DPPH-H, as shown in Figure 4.

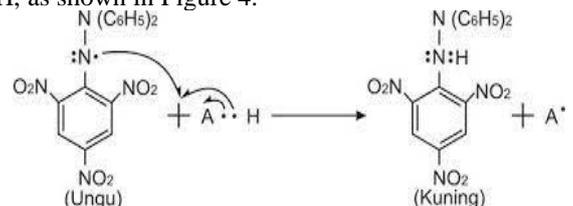


Figure 4. Antioxidant Hydrogen Atom Release Reaction Against DPPH Reagen

Based on Agustin and Gunawan's research (2019), *timun krai* (*Cucumis Sativus L.*) obtained an IC₅₀ value of 189.26 ppm, which means that its antioxidant activity is feeble [21]. In chili powder as a natural red color shaper, an IC₅₀ value of 213.11 ppm was also found [22]. This number

is much smaller than that in kimchi krai, where the antioxidant activity is even stronger. This is because the salt used for kimchi in the initial soaking process draws water out of the fruits containing soluble solids such as proteins, carbohydrates, minerals, and vitamins essential for lactic acid bacteria [23]. However, the fermentation process will increase antioxidant activity because the lactic acid bacteria produced will form bioactive compounds, one of which is antioxidants [24].

Based on the research, the potential of kimchi krai as an antioxidant food source is considered lacking. This is due to the weak antioxidant activity of fresh krai. Adding salt to the kimchi-making process will also result in water content, protein, vitamins, and carbohydrates degradation. This causes the antioxidant activity to decrease. However, kimchi krai still has potential as a probiotic food judging from the results of total bales, total titratable acid, and pH value.

Conclusion

Based on the data obtained in the study of the effect of fermentation duration on the chemical quality of kimchi krai as a probiotic, it can be concluded that the longer the fermentation time, the lower the pH, the increase in total lactic acid bacteria, TAT, and antioxidant activity. This research was conducted with short-term fermentation with a maximum period of 24 hours with the following results: At 24 hours of fermentation, the best results were obtained with the total amount of LAB received, which was 6.1×10^7 CFU/mL, the highest TAT of 0.62%, and the lowest pH value of 3.85, so this kimchi krai is a product that has the potential as probiotic food. At 24 hours of fermentation, the highest antioxidant activity was obtained with an IC_{50} of 429.86 ppm. Based on the IC_{50} value obtained, it is concluded that the antioxidant activity in kimchi krai is fragile in counteracting free radicals, so it is less recommended as an antioxidant agent.

Suggestion

Based on the research that has been done and the potential of kimchi krai as a probiotic food, further research is needed regarding long-term fermentation and different formulas to enable kimchi krai to counteract free radicals as an antioxidant agent strongly.

References

- [1] Benedicta, Y., et al. (2017). Isolation of lactic acid bacteria from kimchi and their ability to produce antibacterial compounds. *Scripta Biologica* Vol 4 No 3, 165-169.
- [2] Azka, A. B. F., Santriadi, M. T., & Kholis, M. N. (2018). Effect of Salt Concentration and Fermentation Time on the Chemical and Organoleptic Properties of Kimchi. *Agroindustrial Technology Journal*, 02(01), 91-97.
- [3] Vaughn. (1982) . Lactic Acid Fermentation of Cabbage, Cucumber, Olives and Other Produc In Industrial Microbiology. Fourth edition. AVI Publishing Co.Texas.
- [4] Hidayat, N., Irdia, N., & Wike, A.P.D. (2006). Making Prebiotic and Probiotic Drinks. Surabaya: Trubus Agrisarana.
- [5] Arini, L.D.D. (2017). Utilization of Good Bacteria in Making Fermented Foods that are Beneficial for Health. *Biomedicine*.10(1): 1-10.
- [6] Parwata, I.M.O. (2016). Antioxidant Teaching Materials. Bali: Udayana University.
- [7] Werdhasari, A. (2014). The Role of Antioxidants for Health. *Indonesian Medician Biotech Journal*. 3(2): 59-61.
- [8] Sadler GD, Murphy PA. (1998). pH and titrable acidity. Di dalam: Nielsen SS, editor. *Food Analysis 2nd edition*. Kluwer Academic(US): Plenum Publishers.
- [9] Lestari, Cheria. (2017). Effect of Salt Solution Concentration and Fermentation Temperature on the Quality of Radish Kimchi. *Journal of Food Engineering and Agriculture*, Vol.5 No.1. Faculty of Agriculture, University of North Sumatra. Medan.
- [10] Pradani, M. & E. M. Hariastuti. (2009). Utilization of Liquid Fraction of Cassava Starch Isolate as Fermentation Media to Replace Tajin Water in Making Salted Vegetable. Research Report of the Faculty of Chemical Engineering. Diponegoro University. Semarang.
- [11] Fatonah, S., et al. (2009). The Effect of Salt Concentration and Addition of Carbohydrate Sources on the Organoleptic Quality of Salted Mustard Greens Products. Bachelor's Thesis, Department of Food Technology and Nutrition. Faculty of Agricultural Technology. Bogor Agricultural Institute. Bogor.
- [12] Winarno, F. G. dan I. E. Fernandez. (2007). Milk and its Fermented Products. M-brio Press, Bogor.
- [13] Chae, L. W. dan Ta Huang, C. (2009). Modeling of Ethanol Fermentation Using *Zymomonas mobilis* ATCC 10988 Grown on the Media Containing Glucose and Fructose. *Biochemical Engineering Journal*. 4: 217 – 227.
- [14] Yang, Seung-Yoon, Elias M.Savitri. (1988). Get to know Korean culture. Publisher: Nam Yang Sa. Korea.
- [15] Codex Alimentarius Internasional Food Standar. (2017). Standar for Kimchi.
- [16] Tayo, A. & Akpeji, S. (2016). Probiotic Viability, Physicochemical and Sensory Properties of Probiotic Pineapple Juice. *Fermentation*. 2 (20): 1-11
- [17] Silalahi, J. Darshieny, N., Silalahi, C.E. (2018). The Effect Of Storage Condition on Antioxidant Activity of Probiotics in Yogurt Drinks. *Asian Journal of Pharmaceutical and Clinical Research*. 11(12): 281-282.
- [18] Badarinath, A., Rao, K., Chetty, C. S., Ramkanth, S., Rajan, T., & Gnanaprakash K. (2010). A Review on In-vitro Antioxidant Methods : Comparisons, Correlations, and Considerations. *International Journal of PharmTech Research*,1276-1285.
- [19] Amic, D., Beslo, D., Trinajstic, N., & Davidovic. (2003). Structure-radical scavenging activity relationships of flavonoids. *Jurnal Croatia Chem Acta*, 76(1), 55-56.
- [20] Prakash, A. (2001). Antioxidant activity medallion laboratories. *Journal of Analithycal Progres*, 19(2), 1-4.
- [21] Agustin, V., dan S, Gunawan.2019. Phytochemical Test and Antioxidant Activity of Krai Cucumber

Extract (*Cucumis Sativus L.*). Tarumanegara Medical Journal. 1(2) : 195-200

- [22] Utami, A. K. (2019). Quality of Raw Chili Powder (*Capsicum frutescens*) with Lime (*Citrus aurantiifolia*) and Tamarind (*Tamarindus indica*) Immersion Treatment After Water Blanching. Thesis.
- [23] Frazier, W. C. dan P. C. Westhoff. (1978). Food Microbiology. 3rd Ed. McGraw-Hill Company Ltd., New Delhi.