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Evaluation Of Capsules Preparations With Active Ingredients From Kimchi Powder And Variations Of Katuk Leaf Powder (*Saoropusandrogynous* (L.) Merr.)

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Abstract:
The Covid-19 pandemic has opened up business opportunities for the Indonesian health supplement industry and food industry, one of which is supplements containing probiotics. As a fermented processed product, kimchi contains many vitamins and minerals and is a good source of probiotics for the body. Therefore, kimchi is processed into capsule form through drying and added katuk leaf powder to add nutrients and prolong the viability of lactic acid bacteria (LAB), increasing the shelf life of kimchi, masking the taste and aroma, and reducing distribution and storage problems of kimchi. Kimchi is made by spontaneous fermentation, then dried using a Food Dehydrator (45℃, ±72 hours). The dried kimchi powder is put into the No.0 capsule shell using a semi-automatic capsule filling device. Tests were carried out on the viability of LAB using microscopic, macroscopic, and TPC methods in MRSA media and evaluation on capsule preparations. The highest total LAB yield was obtained in F_{2KTF} (kimchi fermentation with 7% katuk leaf powder) with an average of 8.40 log CFU/mL. The results of the evaluation of capsule preparations obtained an average of the weight diversity test for each formulation are 0.5507; 0.5615; 0.5432; 0.5455; 0.5702 (F_{Ki}; F_{1Ka}; F_{2Ka}; F_{1KTF}; F_{2KTF}), the average disintegration time was ±11 minutes, and in the hygroscopic test there was no significant change, which means that the capsule preparations met the evaluation requirements and increased the shelf life of kimchi, as well as prolonging the viability of LAB in capsule preparations after storage at room temperature for 14 days.
Key Words*:Dried Kimchi, Katuk Leaf Powder, Lactic Acid Bacteria, Fermentation, Capsule***.**

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I. Introduction

According to the Deputy for Supervision of Traditional Medicines, Health Supplements, and Cosmetics for the POM Agency (BPOM), referring to BPOM Regulation No. 17 of 2021 concerning Guidelines for Assessment of Health Supplement Products Containing Probiotics, states that probiotics are a business opportunity for the health and supplement industry. Indonesian food during a pandemic. Until 2021, data on submissions for registration of products containing probiotics as health supplements at the BPOM have recorded a total of 75 products, of which 67 products have received marketing approval with claims of maintaining digestive health. This is based on scientific studies which show that 80% of the immune system is in the digestive tract which can be affected by consuming probiotics (https://www.pom.go.id). One of the fermented food products which is a source of probiotic BAL (Lactic Acid Bacteria) which is good for the body is kimchi.

Kimchi is a traditional Korean ethnic food, generally made from chicory and other beneficial ingredients made by fermentation methods and has been used as a main dish in Korea for more than thousands of years. Kimchi is a naturally fermented vegetable, so it is considered a healthy and safe food for consumption. In addition, the fermentation process makes kimchi have special characteristics, especially in taste and aroma. Kimchi has a sweet, sour and carbonated taste. The type and concentration of salt, fermented lactic acid, and the addition of spices affect the taste of kimchi (Noh *et al*., 2016).

Many studies have stated that kimchi has beneficial properties such as anti-aging, antiatherogenic, antioxidant, antimutagenic, anticancer, fibrinolytic effects, and antidiabetic activities. Recent studies have reported that kimchi additives can enhance the anti-obesity effect at high concentrations by inoculating pure cultures of Weissella koreensis or Leuconostoc (Leu.) citreum (Cui *et al.*, 2015). Kimchi also contains many vitamins and minerals as well as other nutrients needed by the body (Hongu et al., 2017). As a fermented product, kimchi contains probiotics from lactic acid bacteria which are good for the body. A study of the microbial community taken from several samples of Korean commercial kimchi, states that several species of

lactic acid bacteria are present in kimchi, including Wei.koreensis, Lab. sakei, Weissella cibaria, Lab. graminis, Lactobacillus gelidum, Leu. mesenteroides, Leuconostoc inhae, Leu. gasicomitatum, Wei. confusa, and Leuconostoc kimchii (Park *et al*., 2014).

Consuming a lot of products that contain probiotics has many benefits, especially in increasing the immune defense (immune system). Several species of lactic acid bacteria such as Lactobacillus casei and Lactobacillus bulgaricus are known to increase the production of macrophages and phagocytes (Widiyaningsih., 2011). Where macrophages have the function of destroying antigens and handing them over to T lymphocytes because they have Antigen Presenting Cells (APC) and phagocytes have a vital role to fight infection, foreign particles that may enter the body, bacteria, and dead cells or apoptosis (Kresno, 2010).

Apart from the various nutrients contained in kimchi, some people choose to avoid consuming kimchi because of its distinctive and foreign aroma and taste. Therefore, kimchi is a food that is difficult to internationalize. In addition, kimchi, which is a fermented food, often has distribution problems. Improper storage and distribution conditions can cause changes in the taste, aroma, and texture of kimchi. Kimchi stored at room temperature will only last about 3-5 days, whereas at cold temperatures (in the refrigerator) it can last up to 3-6 months. However, the duration of kimchi storage affects the acidity of kimchi, where the longer the storage duration the higher the acidity level (E. J. Kim *et al*., 2020; M. J. Kim *et al*., 2020)). To overcome this problem, kimchi is processed through drying and made into powder and then made into capsule preparations which will later be used as health supplements.

The capsule dosage form was chosen to cover the distinctive aroma and taste of kimchi, as well as to facilitate storage and distribution. The addition of katuk leaf powder (Sauropus androgynous (L.) Merr.) to the kimchi formulation in this study was carried out as an effort to increase the nutrition in kimchi where katuk leaves have been widely used by Indonesian people for therapy or treatment because they have many vitamins and nutrients (Tiara and Muchtaridi., 2018). In addition, the addition of katuk leaf powder is also expected to extend the viability of lactic acid bacteria in kimchi in dry conditions. The purpose of this study was to ensure that kimchi capsule preparations with variations of katuk leaf powder still contained live lactic acid bacteria and met the requirements for evaluation of capsule preparations.

II. Material And Methods

Research method consists of (7) seven stages, namely preparation of tools and materials, making kimchi, evaluating kimchi, making samples, drying, evaluating dry samples, filling capsule shells and evaluating capsule preparations. The process of making kimchi begins with chopping and washing the chicory in running water, sprinkle with bamboo salt 2.5% of the weight of the chicory per unit gram, let it rest at room temperature (24-260C) for 4-6 hours, check the salinity of the mustard greens

White using a salinity meter. Discard the water that diffuses from the chicory to prevent an abnormal increase in water content during the fermentation process (Cho et al., 2015; Lee et al., 2018; Park et al., 2017). Prepare and mix the gochugaru, sliced radish and scallions, mashed ginger and garlic, fish sauce, rice flour paste, and water, and katuk leaf powder, stir until homogeneous. 5 samples were made: K (Kimchi without the addition of katuk leaf powder); F1, F2, F3, F4 (Kimchi added

Katuk leaf powder) with a ratio of 4:5:6:7 per weight of chicory. The process of making kimchi is done by spontaneous fermentation method. For initial storage, kimchi is placed in a tightly closed sterile container at room temperature (25^0C) for 1-2 days to speed up the fermentation process and

Growth of lactic acid bacteria, then stored at low temperature (4-6 0C) for 7-14 days. Control during the fermentation process was carried out to check the pH level of the kimchi (Khasbullah, Mangiring, and Krisnarini 2020; E. J. Kim *et al*., 2020; Nursidika *et al*., 2020).

The five kimchi samples were dried using the dehydration method, by reducing the water content in the kimchi samples. The kimchi sample was coarsely ground using a blender, dehydrated using a Food Dehydrator ARD-PM99 at 400 -450C for 48-72 hours (Fidyasari, Lestari Eka, and In Oktavia 2022). Then the sample was sieved using mesh No. 18 (Farida, Mana, and Dewi 2019). Then, the pH level (pH meter), salt concentration (Argentometric titration), and drying loss (Moisture Balance) were carried out on the five dry kimchi samples.

Examination of LAB viability in kimchi powder was carried out using the bacterial colony method (macroscopic), examination under a microscope (microscopic), and total lactic acid bacteria test. The method of examining bacterial colonies was carried out by making dry kimchi suspension, adapted for 2 hours in de Man Rogosa and Shape Agar (MRSA) media and incubated for 24 hours to grow the microbes. The examination method under the microscope was carried out by dripping the adapted dry kimchi suspension on an object glass, examined under a light microscope using a 50 x magnification lens. Testing for total lactic acid bacteria was carried out by diluting 1 mL of kimchi powder sample in 9 mL of 0.85% physiological NaCl (10^{-1} dilution).

Dilution was carried out continuously until reaching 10^{-6} dilution. Then the samples from the last 3 dilutions were put into 1 mL of each petri dish. Then as much as 15 mL of de Man Ragosa and Shape Agar (MRSA) was carefully put into each petri dish and then homogenized by making a figure eight motion. After the

medium was solid, the plates were incubated for 24 hours at 37oC using an incubator. Number of coloniescounted. The results of the microbiological analysis are reported using the Standard Plate Count (SPC) (Aristya, Legowo, and Al-Baarri 2013; Rohman, Dwiloka, and Rizqiati 2019).

The process of filling kimchi powder into capsule shells was carried out by manual filling method using a capsule filling tool, with capsule shell number 0. Furthermore, evaluation of capsule preparations was carried out by testing 4 parameters, namely weight uniformity test, disintegration time test and solubility test, hygroscopic test, and test dissolution.

III. Result

Kimchi Making Process Analysis Results

In making Kimchi, all raw vegetables and ingredients used are purchased from local supermarkets and Korean supermarkets in Bandung as well as online buying and selling forums. Then the materials that have been prepared are processed through several stages of the process. The process of making kimchi begins with removing the parts of the mustard greens that will not be used, such as damaged mustard leaves, then washing the chicory with running water. After that, the chicory is salted by sprinkling 2.5% w/w of the weight of the chicory per gram of bamboo salt, then allowed to stand at room temperature (24-26 ℃) for ± 4-6 hours. The salting process is carried out with the aim of supporting the fermentation process and reducing the water content in the mustard so that the mustard becomes softer. In addition, the amount of salt used in the salting process will affect the final composition of the metabolite profile in kimchi (Song et al., 2021). In the process of salting chicory, a process called plasmolysis occurs which is the result of osmosis. When the chicory is sprinkled with salt or soaked in a salt solution, the liquid in the chicory cells will be sucked out and cause the cells in the chicory to shrink causing the mustard to soften. This happens because of the concentration of a more concentrated (hypertonic) salt solution to the liquid in the chicory cells (Istiqamah, A and Rauf A., 2016).

Figure III.1(A) The process of salting chicory; (B) The process of draining the chicory after salting.

The pasta seasoning that will be used for the fermentation process is made while waiting for the salting process. The pasta seasoning is made by heating a quantity of rice flour in water until it thickens, after which it is mixed with gochugaru, sliced radish and scallions, crushed ginger and garlic, and fish sauce with a ratio (w/w) of the weight of the mustard greens used. 5 samples were made according to the formulation prepared in Table III. 1.

Figure III.2 Production of rice flour paste and pasta seasoning used for fermentation.

Chicory that has gone through the salting process is then washed and rinsed under running water and then drained. After that, the chicory is covered with the paste spices that have been made before. Make sure that the paste seasoning coats each piece of chicory evenly. The mustard greens that have been seasoned are then put into an airtight jar to then be fermented.

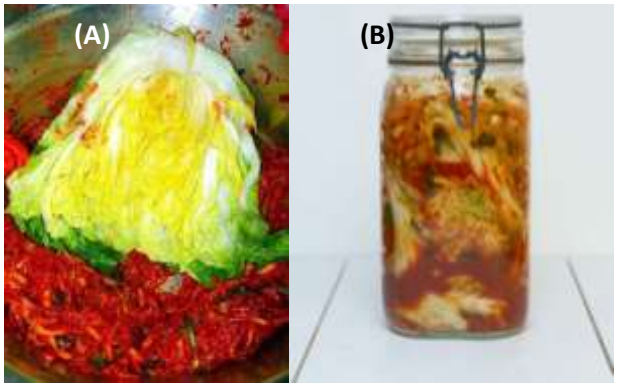

Figure III.3(A) The process of coating Chinese cabbage with pasta seasoning; (B) Kimchi ready to be fermented.

The fermentation process was carried out using the spontaneous fermentation method without using a microbial starter. For initial storage, kimchi is placed at room temperature (24-26 ℃) for 1-2 days to accelerate the fermentation process and the growth of lactic acid bacteria, before storage at low temperature (4-6 ℃) for 7-14 days or until it reaches the value Optimum pH for the continued fermentation process (Khasbullah etc., 2020; E. J. Kim *et al*., 2020; Nursidika*et al.*, 2020).

III.Evaluation of Kimchi

pH Measurement

Measurement of the pH value of kimchi was carried out 3 times, at the initial stage after fermentation, the middle stage during fermentation, and the final stage when the fermentation ended. In the early stages of fermentation, a pH value of 4.97 - 5.36 was obtained. The obtained pH value remained stable with a not very significant decrease to 4.73 - 5.23 in the middle stage during fermentation. A significant decrease in the acquisition of pH value occurred after 14 days of fermentation, the pH value was obtained from 3.98 to 4.12. The optimum pH value of kimchi which is good for consumption is at a pH value of 4.1 - 4.4 (E. J. Kim et al., 2020; M. J. Kim et al., 2020).

Tabel III. 1 Kimchi acidity based on Fermentation Time (Days).

Fermentation (Day)	**Average pH**
1	5.19 ± 0.14^{a}
6	4.99 ± 0.22^{a}
14	4.05 ± 0.53^{b}

Note: Numbers followed by different notation letters (ab) indicate significantly different

Analysis based on data acquisition, fermentation time (days) has a significant effect on obtaining the pH value of kimchi (<0.05) where the longer the fermentation time, the lower the pH value obtained, which means the acidity level in kimchi is higher, this is in line with research (E. J. Kim et al., 2020; M. J. Kim et al., 2020) which showed that the duration of fermentation can affect the acquisition of pH values. The pH value of kimchi on day 1 and day 6 of fermentation showed a significant difference from the pH value of kimchi obtained on day 14 of fermentation.HasilAnalisis Proses Pengeringan

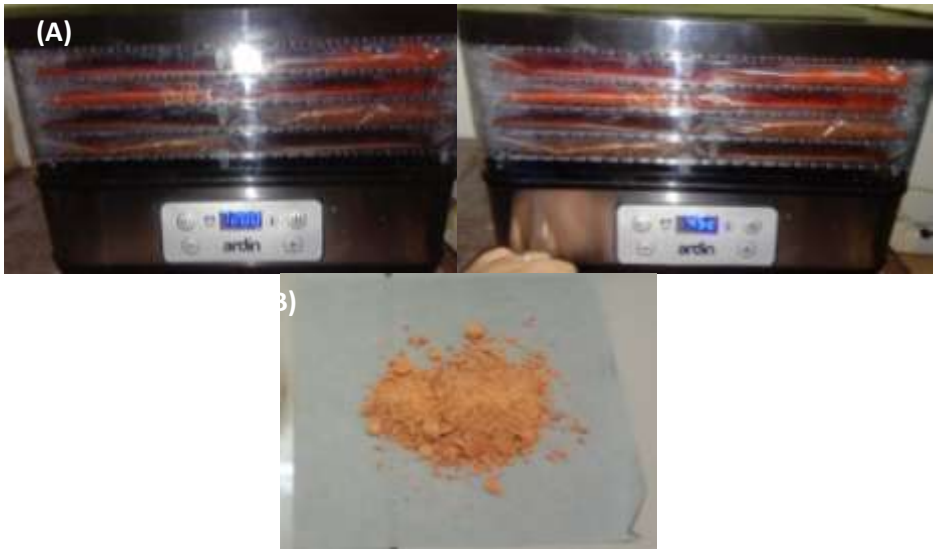

Figure III. 4A) The process of drying kimchi using a Food Dehydrator ARD-PM99 at 45 ℃ for 72 hours; (B) Drying powder.

The temperature used in the drying process is 45 ℃ which is maintained constant to adjust to the optimum temperature for lactic acid bacteria growth of 37 ℃ and can tolerate temperatures up to 45 ℃ (Layadi et al., 2017). The use of temperatures exceeding 45-50 ℃ can damage the survival of lactic acid bacteria. The drying process was carried out for 72 hours. The results obtained after drying are in the form of plates which are then reduced by using a Disc Mill Pulverizer to obtain powder mass. The average dry kimchi obtained was 375.98 g from ±500 g fresh kimchi plus 400 g adsorbent with an average yield calculation of 41.41%. The kimchi powder is then stored in a sterilized airtight container

LAB (Lactic Acid Bacteria) Availability

Microscopic

Testing the viability of lactic acid bacteria microscopically was carried out using the negative staining method, coloring the preparation background so that the bacterial cells would appear transparent under a microscope. The tool used is a light microscope with a magnification of 1000x using Chinese ink as a coloring agent.

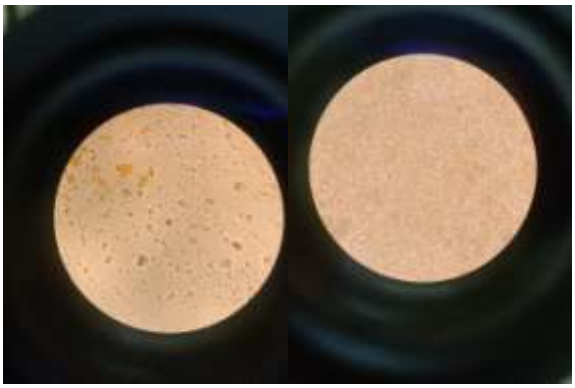

Figure III. 5 Microscopic Observation of Lactic Acid Bacteria (LAB) Viability.

Based on the observations, it can be seen that in each sample there are coccus-shaped or round and clustered bacterial cells, and some cells look like bacilli but not clearly visible. This is because the amount of dye tends to be small, so the observed bacterial cells do not look transparent.

Tabel III. 2 The viability of Lactic Acid Bacteria (LAB) was observed under a light microscope with a magnification of 1000x

.Microscopic LAB viability				
Sample				
FKi	**F1Ka**	**F2Ka**	**F1KTF**	**F2KTF**
+	+	+	+	+

Information :
FKi = Kimchi without Katuk Leaf Powder
F1Ka = Kimchi with 6% Katuk Leaf Powder, Katuk Leaf Powder is added after drying
F2Ka = Kimchi with 7% Katuk Leaf Powder, Katuk Leaf Powder is added after drying
F1KTF = Kimchi Fermentation with 6% Katuk Leaf Powder
F2KTF = Kimchi Fermentation with 7% Katuk Leaf Powder

In this test, 2 grams of dried kimchi was suspended using 9 mL of 0.85% physiological NaCl solution and adapted for 2 hours with the aim of reactivating the bacteria that were originally made doman during drying. Then the samples were diluted to 10-6 dilutions each using 9 mL of 0.85% physiological NaCl solution. Dilution is carried out so that the next test can be carried out, namely the Total Plate Count (TPC) Test. The media used in this test is MRSA media which is a specific medium for maintaining and growing certain bacteria such as lactic acid bacteria.

Macroscopic *Pour Plate*

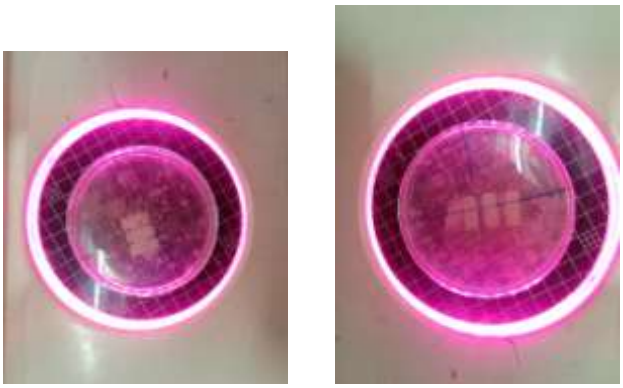

Figure 3. 6 Microscopic Observation of Lactic Acid Bacteria (LAB) Viability with Pour Plate Method

The MRSA medium needed to make the media is 31 grams in 500 mL of sterile water. MRSA media was sterilized using the wet heat method using an autoclave at 121 ℃ for 15 minutes. Bacterial viability was indicated by the presence of white dots on MRSA media after 24 hours of incubation and compared to controls. The control used was MRSA media without additional samples.

Tabel III. 3 Viability of Lactic Acid Bacteria (LAB) observed macroscopically or observed the formation of bacterial colonies in MRSA-specific media for 14 days with an observation interval of 2 days.

Macroscopic BAL viability					
Day-	**Sample**				
	F_{Ki}	**F_{1Ka}**	**F_{2Ka}**	**F_{1KTF}**	**F_{2KTF}**
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+

Information :
FKi = Kimchi without Katuk Leaf Powder
F1Ka = Kimchi with 6% Katuk Leaf Powder, Katuk Leaf Powder is added after drying

F2Ka = Kimchi with 7% Katuk Leaf Powder, Katuk Leaf Powder is added after drying
F1KTF = Kimchi Fermentation with 6% Katuk Leaf Powder
F2KTF = Kimchi Fermentation with 7% Katuk Leaf Powder

The results obtained showed that there was bacterial growth in the media cup containing the sample, while in the control plate there was no bacterial growth indicating that the test technique was carried out aseptically and no contamination occurred. Tests for each sample were carried out three times (triplo).

***Total Plate Count* (TPC).**

The Total Plate Count test was carried out to find out how many lactic acid bacteria there are in log CFU/mL sample units. The test was carried out as a follow-up to the macroscopic viability test of bacteria. Of the six dilutions, the last three plates were selected to be observed and the number of colonies counted using a plate counter. In a petri dish, two transverse lines repel each other so that there are 4 parts in one petri dish. From the three plates of each sample, one plate was taken with the number of bacterial colonies that met the requirements for calculation, namely in dishes with a total of 30-300 colonies (M. Y. Jung et al., 2018). The test was carried out for 14 days with an observation interval of 2 days.

The results obtained showed the addition of colonies formed for each formulation for each observation with an average number of bacteria obtained in log CFU/mL units for each formula was FKi 8.12; F1Ka 8.20; F2Ka 8.34; F1KTF 8.28; and F2KTF 8.40 (See Table 3. 15). Generally, the total lactic acid bacteria in kimchi is in the range of 108-109 (K. Y. Park et al., 2017; Song et al., 2021).

The data obtained was then processed using the Kruskal-Wallis method. The Kruskal-Wallis method was chosen as an alternative to the One Way ANOVA method, used because the distribution of the data obtained was not normally distributed and homogeneous.

Tabel III. 4 LAB (Lactic Acid Bacteria) viability (log CFU/mL)

Fomulation	**Average LAB (Lactic Acid Bacteria) viability (log CFU/mL)**
$\mathbf{F_{Ki}}$	8.12 ± 0.24^{b}
$\mathbf{F_{1Ka}}$	8.20 ± 0.18^{ab}
$\mathbf{F_{2Ka}}$	8.34 ± 0.11^{ab}
$\mathbf{F_{1KTF}}$	8.28 ± 0.13^{ab}
$\mathbf{F_{2KTF}}$	8.40 ± 0.05^{a}

Note: Numbers followed by different notation letters (ab) indicate significantly different.

Based on data acquisition, there is a significant difference (<0.05) between the number of lactic acid bacteria in log CFU/mL units of each formulation. This means that katuk leaf powder added to the formulation significantly affects the number of lactic acid bacteria in log CFU/mL units. The distribution of data based on the average of each formulation shows the result that F2KTF is the best formulation with a subset value of 8.40. The increase in total lactic acid bacteria in the formulation added with katuk leaf powder occurred because there was availability of sources of nutrition and food in the katuk leaf powder media to support the growth of lactic acid bacteria (Kadita et al., 2016)

Capsule Filling

The dried kimchi is ready to be put into the capsule shell. In this study, the capsule shell used was number 0 with a weight of ±500 mg. Capsule shell filling is done semi-automatically with a capsule filling tool.

Figure III. 7 The process of filling capsule shells no. 0 using a semi-automatic capsule filling tool.

The first step is to prepare the capsule shell as needed, then separate the capsule body and head. Close some of the holes from the capsule filling tool until there are 50 holes left. Insert the capsule body into each hole on the tool. After that, pour the sample powder over the tool and then flatten it until all the capsules are full and solid. The capsule head is attached to the tool cover and then pressed against the capsule body until the capsule is completely closed. The capsule which already contains the sample is then cleaned with a sterile cloth before being put into a capsule bottle which is added to a silica bag as a preservative. Repeat the steps five times, where each formulation is made of 50 capsules.

Evaluation of Kimchi Capsules

Variation of Capsule Weight

This test is carried out to ensure that the amount of active substance is distributed homogeneously in each capsule. According to the Ministry of Health of the Republic of Indonesia, 2020, the diversity test of capsule weights was carried out by weighing 10 capsules using a digital scale, then recording and calculating were carried out to ensure that each capsule weight met the requirements as shown in Table IV. 1. Based on the research that has been done, of the 10 capsules tested from each sample, not a single capsule has passed the upper and lower limits of acceptance, meaning that the 10 capsules tested have met the requirements and no need to repeat the test.

Table III. 5 The results of the capsule weight diversity test from an average of 10 capsules compared to the requirements of L1 15.0% and L2 25.0%.

Sample	Average capsule weight	Requirements			
		L1		L2	
		Up border	Down border	Up border	Down border
F_{Ki}	0.5507	0.6333	0.4681	0.6884	0.413
F_{1Ka}	0.5615	0.6457	0.4773	0.7019	0.4211
F_{2Ka}	0.5432	0.6247	0.4617	0.679	0.4074
F_{1KTF}	0.5455	0.6273	0.4637	0.6819	0.4091
F_{2KTF}	0.5702	0.6557	0.4847	0.7128	0.4277

Information :

FKi = Kimchi without Katuk Leaf Powder
F1Ka = Kimchi with 6% Katuk Leaf Powder, Katuk Leaf Powder is added after drying
F2Ka = Kimchi with 7% Katuk Leaf Powder, Katuk Leaf Powder is added after drying
F1KTF = Kimchi Fermentation with 6% Katuk Leaf Powder
F2KTF = Kimchi Fermentation with 7% Katuk Leaf Powder

Disintegration Time Test

Disintegration time testing is carried out with the aim of knowing how long it takes for a capsule or tablet preparation to completely disintegrate in the body in order to give a therapeutic effect. In this test, the capsule will break down into smaller particles so that they can be absorbed by the body. Capsules are declared completely disintegrated if no mass remains in the test equipment or only masses that have a core or an unclear shape are left behind. Based on the test results, the average disintegration time of the tested capsules was ±11 minutes. Based on (Ministry of Health RI, 2020), the disintegration time requirement for hard capsules, if not stated otherwise, is 15 minutes, which means that the five formulations tested met the acceptance requirements for the disintegration time test. Following is the acquisition of data from the results of the disintegration time test.

Tabel 3. 6 Disintegratiosn test

Sample	Disintegration time	
	Minutes	Seconds
F_{Ki}	11	42
F_{1Ka}	10	58
F_{2Ka}	11	56
F_{1KTF}	11	19
F_{2KTF}	10	37

Information :
FKi = Kimchi without Katuk Leaf Powder
F1Ka = Kimchi with 6% Katuk Leaf Powder, Katuk Leaf Powder is added after drying
F2Ka = Kimchi with 7% Katuk Leaf Powder, Katuk Leaf Powder is added after drying
F1KTF = Kimchi Fermentation with 6% Katuk Leaf Powder
F2KTF = Kimchi Fermentation with 7% Katuk Leaf Powder

Table V. 7 Summary of Evaluation Results for Kimchi Capsules

Parametre	Standard/Requirements	Ideal	Not Ideal
Weight uniformity	L1 15.0 % L2 25.0 %	✓	-
Disintegration Time	±15 menit	✓	-
Higroscopic	None significant change	✓	-

IV. Conclusion

Based on data analysis from the results of research that has been done, it can be concluded that:
1. Kimchi capsule preparations still contain live lactic acid bacteria.
2. Preparation of kimchi capsules can extend the shelf life of kimchi.
3. The addition of katuk leaf powder can extend the viability of lactic acid bacteria in kimchi.
4. The kimchi capsule preparation meets the evaluation requirements for the capsule preparation.

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