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The influence of variations in starter concentration *Lactobacillus plantarum* AS4 indigenous from human breast milk to the characteristics of fermented milk

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KEYWORDS

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Lactobacillus plantarum AS4

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Functional food

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ABSTRACT

The fermentation of milk by *Lactobacillus plantarum* AS4 can be classified as a functional or nutraceutical food. *Lactobacillus plantarum* AS4 is a group of Lactic Acid Bacteria (LAB) isolated from human breast milk, which has the ability as a probiotic in vitro. This research aimed to determine the effect of *Lactobacillus plantarum* AS4 starter concentration on the characteristics of fermented milk produced. Variations concentration starter used were 0, 2, 4, 6, and 8% with an incubation temperature of 37 °C for 24 hours. Fermentation parameters collected were probiotic viability, total bacteria, titrable acidity, lactose, protein, and fat content. The results showed that variations in concentration starter *Lactobacillus plantarum* strain AS4 significantly affected the characteristics of fermented milk based on the parameters collected. The viability of fermented milk probiotics increased in milk treated with an 8% starter concentration variation (9.91 ±0.02 log CFU/mL) and total bacteria (8.84 ±0.07 log CFU/mL) in the fermented milk product. The levels of lactic acid (1.66 ±0.06%) and protein (4.32±0.03%) were also increased in fermented milk products with a starter variation of 8%. Significantly, fermented milk products with concentrations starter 8% decreased the pH value (4.10 ±0.01), lactose content (2.33±0.01%), and fat content (2.30±0.05%).

Introduction

Public awareness of the importance of maintaining health is increasing, especially in the current state of the Covid-19 pandemic. The main supporting factor in forming the body's immunity is derived from food intake, which shows that food is a basic need for the survival of human life. The increasing level of health awareness encourages the occurrence of multifunctionality of food to meet the nutritional needs other health implications. Wu et al. (2022), in its development, the health implications generated by food consumption have become an essential consideration in addition to the nutritional content and level of delicacy. Such conditions demand another function of food as the fulfillment of bare life, namely the existence of functional properties of diversified food-processed products. Based on this, the concept of functional foods has been recognized, which is the focus of development to provide optimal health implications. Various foodstuffs of vegetable and animal origin can be used as raw materials to manufacture functional food, animal products such as milk.

Milk is a raw material of animal origin that is easily damaged, so post-harvest processing is necessary. In addition, the relatively high lactose content in fresh milk is an obstacle for people with lactose intolerance to consume fresh milk. The high lactose content in milk can be reduced through further processing, fermentation. Therefore, developing processed cow's milk products through fermentation to produce functional or nutraceutical food products is essential. This functional food product is known as fermented milk (Savaiano and Robert, 2021). Pereira and Rodrigues (2018) defined fermented milk as a dairy product fermented by probiotic bacteria activity. This dairy product contains probiotics that benefit the digestive tract because they can improve intestinal microflora balance and can survive gastric acidity to occupy the intestines in large enough quantities. Pereira and Rodrigues (2018) also say that various kinds of

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beverage fermented milk products resulting from the probiotic bacteria activity are publicly well-known, including yogurt, acidophilus milk, bulgaricus milk, kefir, forehead (from India), hamao (from Central Asia), Yakult, etc.

Today, the functionality of fermented milk products has begun to be widely developed, by utilizing the role of LAB, which has the ability as a probiotic in vitro. Probiotics are defined as live microbes that are added to food for dietary needs and provide health effects for the host by improving the intestinal microflora balance (Li et al., 2019). According to WHO/FAO, probiotics are live microorganisms that can benefit human health when administered in sufficient quantities (Motevaseli et al., 2017; Lebeer et al., 2018). The International Scientific Association of Probiotics and Prebiotics (ISAPP) stipulates that probiotics must be alive when administered, have health benefits, and be administered effectively (Hill et al., 2014).

The condition for a food to be called a probiotic food, the number of living cells is generally required to be at least 6 or 7 log CFU/mL or g. Similar numbers are also valid for yogurt acteria. These values are accepted in various national and regional regulations (De Simone, 2019 ; Kycia el al., 2020). Buriti et al. (20015) also stated that in addition to the cell count of the probiotic food per g or mL, the consumption amount is also important to achieve therapeutic effects. A minimum therapeutic daily dose of 8–9 log CFU, corresponding to 100 g of a food product containing 6–7 log CFU/g, has been suggested to induce beneficial probiotic effects. The influence of fermented milk products on the gut microbiota depends on the type of fermented product and the bacterial strain used, the number of bacteria and the time of supplementation.

The development of functional foods in a country is not only beneficial for consumers due to their health or nutritional benefits, but also an opportunity for the food industry and benefits the government. The role of probiotics is now starting to be widely used for the development of functional food products. For the food industry, the high demand for functional food means an opportunity to increase profits by innovating product development and food formulations according to market demand. Currently, most probiotic isolates come from abroad, impacting the high price of probiotic food products. Thus, it is necessary to explore local probiotic isolates native to Indonesia and indigenous to human origin. One of the potential sources of probiotic isolation can be obtained from breast milk (ASI). Breast milk is one source of LAB isolation, which functions to maintain the balance of the digestive tract microflora and boost the immune system of newborns. LAB as a probiotic has high viability in the digestive tract if it comes from the human body (Luz et al., 2021).

Exploration of LAB isolates from humans that have potential as probiotics have been widely carried out. Widodo et al. (2012) have isolated and selected LAB from baby fecal material that has the potential as probiotic agents, including the Lactobacillus and Pediococcus genera. Thus, it can be argued that, LAB in breast milk have the potential as probiotics. This is confirmed by the results from Nuraida et al. (2011). They have evaluated in vitro the ability of LAB isolates from breast milk to assimilate cholesterol and deconjugate bile salts. The test results showed that *Pediococcus pentosaceus* 1-A38 was the most potential isolate to be used in developing probiotic products with specific functional properties for lowering cholesterol by assimilation and deconjugation of bile salts. The same study was conducted by Serrano-Nino et al. (2016), who isolated LAB from breast milk four months after birth. The selected LAB isolates showed that *Lactobacillus fermentum* JCM3 had the ability as a probiotic. Anindita et al. (2018) have also successfully isolated 13 LAB isolates with the potential as probiotics in vitro. The isolates consisted of *Lactobacillus paracasei*, *Lactobacillus case*s, *Pediococcus acidilactici*, *Lactobacillus plantarum*, and *Weisella confusa*.

Lactobacillus plantarum is a probiotic bacteria that can play a role in developing fermented milk products. This is reinforced by Liu et al. (2018) that the bacteria commonly used as a source of probiotics mostly come from the LAB group. Several types of bacteria are included in probiotic bacteria, including Lactobacilli (i.e., *Lactobacillus casei*, *Lactobacillus plantarum*), Bifidobacteria (i.e., *Bifidobacterium bifidum*, *Bifidobacterium breve*). Narzary et al. (2016) also added that fermented foods are produced by using lactic acid bacteria (LAB) comprised of a number of genera (e.g., *Pedicoccus, Lactobacillus, Streptococcus, Lactococcus, Leuconostoc, Enterococcus*, etc.) that yield lactic acid as a major metabolite. The fermentation is carried out by bacteria, yeast, filamentous fungi or a combination of these. Zheng et al (2020) stated that but it has recently been proposed to merge *Lactobacillus* and *Leuconostoc* in one family Lactobacillaceae. The genus Lactobacillus was also reclassified into 25 genera.

Urbaya et al. (2017) stated that *Lactobacillus plantarum* has probiotic characteristics such as acid resistance. *Lactobacillus plantarum* has positive implications useful in forming lactic acid, the highest hydrogen peroxide producer compared to other LABs and also produces bactericidal bacteriocins. *Lactobacillus plantarum* can be used as a starter in fermentation, increasing lactic acid production. Collado et al. (2010) reported that the mechanism of *Lactobacillus plantarum* in fighting pathogenic bacteria are as follows: 1) The presence of antimicrobial agents against pathogens, 2) The process of immunomodulation (adjustment of the immune response so that it reaches the desired level), 3) Improvement of the protective function, 4) Attachment: competition in inhibiting pathogens, inhibiting and occupying the attachment site of pathogens, and 5) Aggregation and coaggregation with pathogens. According to Leeber et al. (2018), fermented milk's quality is determined by the total solids contained in milk, raw materials, starters, high protein content, and low syneresis rates. During fermentation, carbohydrates, proteins, fats, and nucleic acids are broken down into simpler components and affect the flavor and texture of foodstuffs. The first components bacteria attack are carbohydrates (lactose), protein, and fat.

Microbiological and chemical characteristics of fermented milk products can be influenced by adding a variety of probiotic starters. This study aimed to determine the effect of starter variation of *Lactobacillus plantarum* AS4 indigenous from breast milk on the characteristics of fermented milk, including microbiological and chemical characteristics. There is a change in the characteristics of dairy products during the fermentation. There may likely be an increase in the number of probiotic bacteria, acidity value equivalent to lactic acid, and protein content, as well as, a decrease in total bacteria, pH value, lactose content, and fat content. The results of this study are expected to increase food diversification of processed cow's milk products as a functional food by utilizing the LAB group as indigenous local probiotics from breast milk. In addition, it is expected to unlock the potential of local probiotics, especially those from breast milk, in the development of functional food products.

Materials and Methods

Material

This study used an isolate of the probiotic bacterium *Lactobacillus plantarum* AS4 obtained from the isolation results in previous studies (Anindita et al., 2018; Anindita, 2022). The source isolation of this probiotic isolate was obtained from breast milk. Fresh cow's milk was used as the essential ingredient for making fermented milk obtained from UPT Dairy Cattle, Faculty of Animal Science. Powdered skim milk is used in the starter manufacturing process. The chemicals used included de Mann Rogosa and Sharpe (MRS) medium (Merck, Germany; 52.2 g/L) with a pH of 6.2, technical agar, distilled water, 70% alcohol, 10% potassium iodide (KI), 2 N HCl, 0.86% NaCl, glycerol, sucrose, NaH_3, concentrated H_2SO_4, Na_2S2O_3, $ZnSO_4$, NaOH. 0.75 N, 0.1 N NaOH, 50% NaOH, 0.02% methyl blue, 0.02% methyl red, 1% PP indicator, Chloramine-T, catalyst and boiling stone (Merck, Germany).

Methods

Sterilization

The sterilization of the equipment was carried out using an autoclave utilizing water vapor for 15 minutes at a temperature of 121°C with a pressure of 1 atm. Thus, faster coagulation occurs in a wet state could kill microorganisms or endospore bacteria by denaturation or coagulation (Rizal et al., 2016).

Re-culture of Lactobacillus plantarum AS4

The starter bacteria *Lactobacillus plantarum* AS4 was taken from the stock culture with wire loops, streaked on a petri dish containing MRSA media, and then incubated at 37 °C for 24 hours. Furthermore, the bacteria were transferred to the media so that it was slanted and then incubated again in the incubator at the same temperature and time. Inoculate *Lactobacillus plantarum* from agar slanted to 7 mL of MRS Broth media and then incubate using an incubator set at 37°C for 24 hours (Nurgrahadi et al., 2020). The culture that has been propagated can then be used to manufacture starters.

Preparation of *Lactobacillus plantarum* AS4 starter

Preparation of starter *Lactobacillus plantarum* AS4 using the method done by Nurgrahadi et al. (2020) with modifications. Liquid media of 10% (w/v) skimmed milk was prepared by weighing 10 g of skim milk powder, dissolved in 100 mL of distilled water, and sterilized at 110°C with a pressure of 13 psi for 10 minutes. *Lactobacillus plantarum* AS4 culture in a liquid medium of as much as 2% was inoculated into 100 mL of skim milk (v/v). After being inoculated with

Lactobacillus plantarum, the mixture was then incubated at 37°C for 20 hours to form a curd, this is called the mother starter. The mother starter was inoculated into sterile skim milk with a volume of 100 mL as much as 5% and incubated at 37°C for 20 hours, and the result was called bulk starter. The bulk starter was then inoculated into the milk to be fermented with several variations in concentration (i.e, 0, 2, 4, 6, and 8% (v/v)).

Preparation of fermented milk

The making of fermented milk referred to the method of Suharto et al. (2021) with modifications. Starter *Lactobacillus plantarum* AS4 is prepared to manufacture fermented cow's milk. Cow's milk was added with 6% skim (w/v) and 3% sucrose (w/v). The mixture was then homogenized and pasteurized at 72°C for 15 minutes and cooled to a temperature that reached 40°C. Furthermore, as many as 0, 2, 4, 6, and 8% (v/v) variations in starter concentration were added into pasteurized milk and then incubated at 37°C for 18 hours. Next, fermented cow's milk was analyzed for microbiological and chemical characteristics.

Analysis of microbiological characteristics

Analyzed microbiological characteristics include the viability of probiotics and total bacteria. This test was carried out with three replications. It calculated viability/total probiotic bacteria using MRS agar media added with 0.15% (w/v) bile salt based on research conducted by Zhang et al. (2016) with modifications. Meanwhile, the total bacteria was calculated using the Total Plate Count (TPC) method to determine the number of bacteria contained in the sample by growing it on MRS media. The total plate number is the number of aerobic mesophyll bacteria that live in the sample. Aerobic mesophyll bacteria are a group of bacteria that grow well at a temperature of 25-40˚C in an acidic atmosphere. Count bacteria by recording the growth of colonies in each dish containing 25-250 colonies and counting the number of bacteria that grow with a colony counter (Wahyu et al., 2012).

Analysis of chemical characteristics

The chemical characteristics analysis include the measurement of pH value, lactic acid content (%), protein content (%), lactose content (%), and fat content (%). This test was carried out with three repetitions. Measure the pH value with the potentiometer method using a pH meter. The fat content was determined using the Babcock method (Hadiwiyoto, 1994). Lactose content was determined by titration to measure the level of lactose in the filtrate. Protein content was determined using the Kjeldahl method (Sudarmadji et al., 1997).

Data analysis

The experimental design used in this experiment was a completely randomized design (CRD). The data obtained were analyzed by ANOVA to determine whether or not there was a difference in treatment. If there was a difference between treatments, it was continued with the DUNCAN test with a significance level of 0.05. Nonparametric data analysis was conducted using SPSS 16.0 (Zein et al., 2019).

Results and Discussion

Microbiological characteristics of fermented milk

The microbial characteristics of fermented milk are shown in Table 1. The results of statistical analysis showed that variations in starter concentrations significantly affected the number of live probiotics (viable) in fermented milk (P<0.05). During the fermentation, the number of probiotics in fermented milk increased. Addition of starter at all concentrations were able to increase the total probiotics (P<0.05) in the fermented milk. The test results on the number of live probiotics (viable) in fermented milk were linearly proportional to variations in starter concentration.

Table 1. The results of the analysis of microbiological characteristics (log CFU/mL) of fermented milk

Variation of starter concentration (%)	Microbiological analysis of fermented milk (log CFU/mL)	
	Probiotic viability of *Lactobacillus plantarum* AS4	Total bacteria
0	$0.00±0.01^{a}$	$7.09±0.04^{a}$
2	$8.34±0.06^{b}$	$7.53±0.05^{b}$
4	$8.71±0.04^{c}$	$8.12±0.03^{c}$
6	$9.20±0.05^{d}$	$8.95±0.01^{d}$
8	$9.91±0.02^{e}$	$8.84±0.07^{e}$

Remarks: $^{a, b, c, d, e}$ Different superscripts in the same row and column showed significant differences (P<0.05)

The higher concentration of starter used increased the number of live probiotics (viable) in fermented milk. It can be seen that the average increase in the number of live probiotics (viable) is 0.40 to 0.70 log CFU/mL with the same incubation time. Based on Table 1, it can be seen that the increase in total probiotics in fermented milk was influence by the concentrations of starters. The highest increase in total probiotics (0.70 logs CFU/mL) was found in fermented milk products with addition of 8% starter. According to Mills et al. (2011), *Lactobacillus plantarum* can inhibit pathogenic microorganisms in foodstuffs with a high zone of inhibition compared to other LABs. Thus, the type of probiotic bacteria, when applied to fermented milk products, may increase the use-value, which has health implications for the product. Further stated by Mills et al. (2011), *Lactobacillus plantarum* showed good effectiveness to suppress the growth of *Listeria innocua* in making cheese with pasteurized milk as the base material without the addition of nisin.

The increase in the population of probiotics during fermentation occurred, was likely influenced by the availability of nutrients in the media consisting of powdered skim milk, glucose, and fresh milk. Using skim milk powder as a mixture in the ingredients for making fermented milk provides a supply of nutrients, (i.e., protein and lactose), which will be used as substrates during fermentation. Crowley et al. (2016) stated that skim milk powder is used as a source of dairy proteins in several food formulations, including beverages, confectionery, soups, sauces and desserts, wherein the dairy proteins for example provide gelation, emulsification or foam formation. Converting milk solids into powder prolongs the shelf-life of milk and enables storage for long time at ambient temperatures by a combined action of thermal treatment during processing and reduced water activity. In addition, in most applications, the skim milk powders are rehydrated in water prior to use. Similarly, Sanlier et al. (2016) also found that the composition of the substrates used and the fermenting microorganisms are the major factors that influence fermented food such as glucose. Glucose as the primary carbon source in metabolic processes and can be fully utilized by LAB Moreover, food treatment and the length of fermentation during processing may also affect food fermentation. Nuraida et al. (2011) added that the presence of fiber in the media is also used specifically and selectively by probiotic bacteria. The fiber used by probiotic bacteria may not necessarily be used by other probiotic bacteria, depending on the ability to produce enzymes to metabolize fiber.

Rai and Bai (2015) further stated that the viability of probiotics in food products is influenced by storage temperature factors, oxygen availability, pH, and the presence of microorganism competitors. The bacterial culture's compatibility also influences the bacteria's viability with the substrate. The compatibility of probiotic bacteria with the substrate was evaluated using organoleptic, microbiological, and chemical parameters in the finished probiotic beverage product. In addition, Gallina et al. (2019) stated that viability is also influenced by the probiotic strain used, interactions between species, culture conditions, fermentation time, storage conditions, sugar components, and nutrient availability.

The calculation of the viability of this probiotic uses MRS Agar media with the addition of 0.15% bile salt (B-MRS). Bile salt is one of the obstacles for LAB, which is not a probiotic strain when it reaches the digestive tract, so the calculation with the addition of bile salt is counted as probiotic bacteria. The fermentation process with variations in starter concentration caused a significant increase in total probiotics ($P<0.05$). The results showed that fermented milk from all concentration met the standard of yogurt products as functional food. In agreement with Lahteinen et al. (2010), that the benefits of probiotics could be obtained when consuming foods which contain probiotics 10^6-10^7 per mL or g.

Table 1 shows that variations in starter concentration affect the total bacteria in fermented milk products. Total bacteria at starter concentrations of 0, 2, 4, and 6% showed a linear increase with an average value of 0.4 - 0.8 log CFU/mL. Furthermore, the total bacteria decreased by 0.09 log CFU/mL in fermented milk products with a starter concentration of 8%. This is due to the decreasing environmental pH conditions followed by a decrease in the availability of nutrients in the media. Mengesha et al. (2022) said that pH, substrate concentration, temperature, microorganisms, fermentation time and water activity the most important factors for fermentation process. In line with previous research by Yunus et al. (2015), that too short lactic acid fermentation time may cause LAB growth is not optimal. The population is less to be categorized as probiotics. At the same time, too long fermentation time may result in a too sour taste in the product. Also, it may cause a decrease in the LAB population due to depletion of nutrients in the product and the accumulation of toxic metabolites (i.e., ethanol produced by heterofermentative LAB).

Jaishankar and Preeti (2017) added that as the pH values of the LAB begin to decrease with high cell concentrations. The entry of bacteria to the stationary phase can be caused by different factors, including limitation of a specific essential nutrient, accumulation of toxic by-products, presence of stress factors such as changes in pH, temperature, osmolarity, etc. As the cell enters this phase, there is a reduction in cell size and the DNA/protein ratio is said to increase during transition to stationary phase. Stationary phase survival is a means of bacterial adaptation by which bacteria survive under conditions of stress or starvation.

Chemical characteristics of fermented milk

Chemical characteristics, including pH value, titratable acidity content (%), lactose content (%), protein (%), and fat (%), were also measured against variations in the concentration of starter fermented milk products. The average chemical characteristic test results can be seen in Table 2.

a. *Acidity value (pH)*

The acidity value (pH) indicates the level or degree of acidity of a product. Thus, the lower the pH value, the higher the acidity of the lactic fermented beverage product. Table 2 shows that pH value of fermented milk decreased with an increase in the starter concentrations. LAB's activity decreases pH of fermented milk likely due to breaking of lactose into lactic acid. Li et al. (2021) stated that the production of lactic acid due to sugar metabolism causes a decrease in the pH of yogurt. This is related to the increasing number of LAB that uses lactose. The more sugars metabolized, the more organic acids are produced, thus, pH automatically. This agrees with Xiang et al. (2019) that the main factors contributing to food nutritionals value include its digestibility and the number of vital nutrient present. Both nutrients, as well as digestibility, may be improved by the the process of fermentation and an increase in nutrients availability was parallel to an increase in bacterial cells. This may also impact the maximum sugar metabolism, leading to increase the total acid and decrease the pH value.

Table 2 also shows that the starter concentration variations significantly affected the pH value of fermented milk (P<0.05). The fermented milk products had the lowest pH value (4.10 ±0.01) at a starter concentration of 8% (v/v). The pH value decreased from 0.4 to 0.5 in each concentration variation treatment. The decrease in pH indicates the breaking down of lactose during fermentation to produce acids, especially lactic acid. Table 2 shows a positive correlation between the pH value and lactic acid production; the lower the pH value, the more lactic acid production. The decrease in pH during fermentation was possibly due to the accumulation of lactic acid as more homofermentative bacteria were formed. Homofermentative *Lactobacillus* produces 90% of lactic acid from glucose metabolism. A decrease in pH correlated with an increase in total acid, possibly due to an increase in the amount of *Lactobacillus plantarum* AS4 used for fermentation.

This result is well within the results from Lengkey et al. (2013) that the pH value was lower when the starter concentration was higher. They found that increasing starter concentration was correlated with an increase in the acidity level. The pH value can be obtained because there is acid content from the starter bacteria in the sample, in which a lower pH value was generated from samples with a higher starter concentration. Terefe and Augustin (2020) stated that during fermentation, the microbial metabolism enhances the macromolecules' digestibility and improves the bioavailability of macro/micronutrients and phytochemicals. For the removal of antinutrients, allergens and toxins, fermentation is considered as one of the most effective processing methods. During fermentation a decrease in pH causes the taste to become more acidic due to the formation of lactic acid as the main product of LAB metabolism.

Table 2. The Results of the analysis of chemical characteristics of fermented milk

Variation of starter concentration (%)	Analysis of chemical characteristics				
	Acidity Value (pH)	Titratable acidity (%)	Lactose content (%)	Protein content (%)	Fat content (%)
0	6.00 ±0.04^{a}	0.27 ±0.05^{a}	4.53 ±0.03^{a}	3.08 ±0.02^{a}	3.70 ±0.03^{a}
2	5.50 ±0.01^{b}	0.79 ±0.03^{b}	4.07 ±0.04^{b}	3.27 ±0.03^{b}	3.20 ±0.04^{b}
4	5.10 ±0.03^{c}	0.97 ±0.06^{c}	3.57 ±0.04^{c}	3.47 ±0.05^{c}	2.90 ±0.02^{c}
6	4.50 ±0.05^{d}	1.33 ±0.02^{d}	3.15 ±0.02^{d}	3.79 ±0.07^{d}	2.70 ±0.04^{d}
8	4.10 ±0.01^{e}	1.66 ±0.06^{e}	2.33 ±0.01^{e}	4.32 ±0.03^{e}	2.30 ±0.05^{e}

Remarks: $^{a, b, c, d, e}$ Different superscripts in the same row and column showed significant differences (P<0.05)

The formation of lactic acid causes the milk to become sour, and the pH to decrease. In addition, the higher starter concentration indicates the number of bacteria that have activity in producing organic acids (i.e., lactic acid, acetic acid, and propionic acid). The number of bacteria that can produce organic acids causes the acidity of the lactic acid equivalent of fermented milk products to increase, thus decreasing the pH value. This indicates that the starter concentration affects the fermented milk's pH value. Thus, the increase in the number of live probiotics and the number of LAB's positively correlates to the increase in starter concentration. The decrease in the pH value of fermented milk products negatively correlated with the different concentrations of the starter used and the increase in the number of live probiotics and bacteria (Table 1).

b. ***Titratable acidity***

The statistical analysis showed that variations in the concentration of starter *Lactobacillus plantarum* AS4 had a significant effect (P<0.05) on the titratable acidity of fermented milk. A positive correlation was seen between an increase in starter concentration, the number of bacteria, and titratable acidity during the fermentation. The titratable acidity increased with increasing starter concentration, where the highest titratable acidity (1.66±0.01%) was from fermented milk with a starter concentration of 8%. The content of lactic acid formed increases with the higher concentration of starter, thus, the sour smell becomes more pungent. Karimi et al. (2015) reported that the acetyl-CoA pathway mainly converted lactic acid formed in fermentation into acetic acid, propionic acid, and butyric acid. Incubation time and starter concentration might affect the formation of lactic acid.

The results show that titratable acidity of fermented product from all treatments was meeting with the Codex (2011) and SNI 01-2891-2009 standards of 0.3%, and 0.5 - 2.0% (w/w), respectively. The production of organic acids, namely lactic acid, is influenced by the availability of media as a carbon source. Skim milk is one of the carbon sources in the manufacture of fermented milk. Similarly, Triyono (2010) stated that skim milk used in the making of fermented milk acts as a carbon source because it contains lactose used by microbes to form cell materials and obtain energy.

Table 2 shows that the increase in titratable acidity negatively correlates to the pH value of fermented milk products. This is also explained by Suharyono and Muhamad (2011,) that a decrease in pH was due to the fermentation process of carbohydrates, glucose, and lactose, which produces lactic acid by LAB. Also described by Utami et al. (2010) that a decrease in pH balanced the increase in lactic acid in fermented milk. Therefore, the greater the value of lactic acid levels, the lower the pH value. The increase in lactic acid accumulation may affect the environment's pH. The lactic acid produced may be secreted from the cells and accumulate in the fermented liquid. Similarly, Rahmawati (2015) stated that lactic acid and acetaldehyde produced from the breakdown of glucose by *Lactobacillus plantarum* bacteria may significantly decrease the pH of the medium or increase the acidity level. Rosiana and Armansyah (2013) also stated that the level of lactic acid in fermented milk products is influenced by the amount and type of starter used.

c. ***Lactose content***

The statistical analysis showed that the treatment with variations in starter concentration significantly affected the lactose content of fermented milk (P<0.05). All treatments with the addition of starters were able to lower lactose content (P<0.05) compared to controls (0%) with the same incubation time. The average values of lactose at concentrations of 0, 2, 4, 6, and 8% were 4.53±0.03, 4.07±0.04, 3.57±0.04, 3.15±0.02, and 2.33±0.01%, respectively. Kennedy (2016) stated that microorganisms are both catabolic and anabolic, break down complex compounds, and synthesize complex vitamins and other growth factors. The fermentation time affects how much lactose that can be broken down. If the fermentation time is too short, this may lead to not optimal utilization of lactose into lactic acid by probiotic bacteria.

The decrease in lactose levels ranged from 0.4 to 0.50 for each treatment with different concentrations. There was a negative correlation between the number of total probiotics and bacteria on a decrease in lactose content of fermented milk. The low lactose content in fermented milk products is an alternative for milk processing consumed by Lactose intolerant sufferers. Furthermore, Liptakova et al. (2017) stated that LAB utilize fermentative carbohydrates to form lactic acid. Based on hexose sugars' utilization, the pathways are divided into two groups. In homofermentative pathways, the sole end or primary product of glucose fermentation by some LAB such as *Streptococcus, Pediococcus, Lactococcus*, and some lactobacilli is lactic acid. In contrast, in heterofermentative pathways, microorganisms such as *Leuconostoc*, *Weisella*, and some lactobacilli, the end products are ethanol, lactate, and CO2.

Also described by Dutra et al. (2015), LAB utilizes sugar as a source of energy and growth and produces metabolites from lactic acid during fermentation. LAB cause the hydrolysis of lactose through the production of ß-galactosidase. Hydrolysis of lactose is vital for lactic acid production, which lowers the bowel's pH, inhibiting the putrefaction microorganisms from growing. Also, lactic acid is necessary for the absorption of calcium and organoleptic properties.

Lactose content in fermented milk product is also influenced by its ingredients such as fresh cow's milk, sucrose, and skim milk. The addition of skim milk powder causes the fermented milk product to have an increased lactose and casein content. This is due to the components of skim milk. Ballard (2013) stated that skim milk contains about 20% dissolved solids (w/v) and the physical properties of skim milk are that it has a maximum water content of 4%, a maximum fat content of 1.2%, and a maximum acidity of 0.11-0.15%. Lactose and casein are indispensable components in fermentation to be metabolized into lactic acid, acetic acid, diacetyl, and alcohol.

d. *Protein content*

Table 2 shows that the statistical analysis indicated that the variation in starter concentration significantly affected the protein content of fermented milk products (P<0.05). The addition of starter at different concentrations increased the protein content to a greater extent (P<0.05) than the control (0%) with the same incubation time. The protein content in this study was obtained using the Kjeldahl method by calculating the total N in fermented milk products. The results showed that increasing starter concentration was parallel to an increase in protein content. The highest protein content in fermented milk was obtained by adding of 8% starter. These values were well within the minimum requirements for protein content of 2.7% based on the Codex (2011) and SNI 01-2891-2009 standards.

The increase in protein content from all treatments was also influenced by the total population of bacteria present in the fermented milk product, both total LAB and probiotics (*Lactobacillus plantarum* AS4). Based on Table 1, whole yogurt and probiotic bacteria were positively correlated with the value of protein content. The number of viable bacterial cells influences protein levels. An increase in viable bacterial cells increases the enzymes used to break down proteins (proteolytic activity) and increase protein synthesis, including protein-breaking enzymes (proteases). Fermented milk with various concentrations of *Lactobacillus plantarum* AS4 increased protein content and the number of viable bacterial cells, both total probiotics and bacteria.

A previous study by Lengkey et al. (2013) stated that during the 2 -hour incubation period, the protein content of fermented milk would increase due to optimum bacterial growth, thereby increasing protein availability. Marangoni et al. (2019) stated that the concentrated drink is a fermented milk product in which the protein has been increased before or after fermentation to a minimum of 5.6%. Fermented milk drinks contain a minimum of 40% fermented milk, as well as other microorganisms, in addition to the specific starter cultures.

Based on the test results, it can be seen that the higher starter concentration caused an increase in protein content in fermented milk. This was due to the increased activity of the proteinase enzyme *Lactobacillus plantarum* AS4 formed during fermentation. This study is supported by Filannino et al. (2014), that the production of proteinase enzymes caused an increase in protein levels during the fermentation of *Lactobacillus plantarum*. The study confirmed that the protein levels increased alongside an increase in the activity of protease enzymes produced by microbes in fermentation. Increase in the protein content was also due to an increase in the amount of cell biomass that acts as a Single Cell Protein (SCP) (also known as a protein obtained from microorganisms). In line with Setiarto and Widhyastuti (2016) that during LAB fermentation, *Lactobacillus plantarum* produces peptidoglycan in the cell wall, composed of glycoprotein and lipoprotein components. The component that increases the protein is also present in the modified tuber flour and often analyzed as a soluble protein.

e. *Fat content*

Table 2 shows that the variation of starter concentration significantly affected the fat content of fermented milk products (P<0.05). After fermentation for 20 hours, the fat content decreased by 0.2 – 0.5%, giving an average value of 3.70 ± 0.03 (control), 3.20 ±0.04 (2%), 2.90

±0.02 (4%), 2.70 ±0.04 (6%), and 2.30 ±0.05 (8%), respectively. This shows that *Lactobacillus plantarum* AS4 could reduce the fat content by being absorbed as an energy source for growth. In addition, Bruggeman et al. (2020) reported that *Lactobacillus plantarum* could synthesize functional fat, for example Short Chain Fatty Acid (SCFA).

Table 2 shows that fermented milk products' fat content decreased with increasing starter concentration. This illustrates that the activity of bacteria in fermented milk products can produce lipase enzymes that play a role in hydrolyzing fat. These results prove that total viable probiotics and LAB in fermented milk products negatively correlated with a decreased fat content. The number of viable probiotics and LAB is related to the amount of lipase enzyme production to degrade fat. Sawitri (2011) added that more bacteria growing in milk leads to more production of lipase enzymes, thus increasing fat hydrolyzation and causing a greater reduction of fat content. The lipase enzyme in milk hydrolyzes the glycerides to release the fatty acids. Lipase in milk comes from bacteria found in milk, mainly if it contains many bacteria. Rizzoli and Biver (2018) stated that the products of the SCFA metabolism could directly modify the local intestinal metabolism, support the function of the intestinal barrier, and modify the pH of the intestine. Such mechanisms may influence the availability of calcium and increases its absorption.

Conclusion

Based on the results, variations in the concentration of starter *Lactobacillus plantarum* AS4 during fermentation affected the microbiological and chemical characteristics of fermented milk products. The increase in the concentration of starter *Lactobacillus plantarum* AS4 during fermentation showed a positive correlation to the increase in total probiotic *Lactobacillus plantarum* AS4, total bacteria, titratable acidity, and protein content in the product. Increased starter concentration of *Lactobacillus plantarum* AS4 negatively correlated during fermentation to decreased pH values, lactose, and fat content in fermented milk products. The characteristics of fermented milk from all treatments were in accordance to requirements the standards of fermented milk based on Codex (2011) and SNI 01-2891-2009.

Declarations

Conflict of interests The authors declare no competing interests.

References

Anindita, N. S., Novalina, D., and Sholikhah, A. N. (2018) *Isolasi dan Identifikasi Bakteri Asam Laktat Asal Air Susu Ibu sebagai Kandidat Probiotik dalam Pangan Kesehatan* (*Isolation and Identification of Lactic Acid Bacteria from Mother's Milk as Probiotic Candidates in Health Food*) Yogyakarta: Laporan Penelitian Dosen Pemula (PDP) 2018. [In Indonesian]

Ballard, K. D. (2013) ‘Low-fat milk ingestion prevent postprandial hyperglycemia-mediated impairmentsin vascular endothelial function in obese individuals with metabolic syndrome’, *Journal Nutrition*, 143(10), pp. 1602-1610

Bruggeman, F. J., Robert, P., Douwe, M., and Bas, T. (2020) ‘Searching for principles of microbial physiology’, *FEMS Microbiology Reviews,* 44 (6), pp. 821-844

Buriti, F.C., da Rocha, J.S., Assis, E.G and Saad, S.M. (2005) ‘Probiotic potential of Minas fresh cheese prepared with the addition of *Lactobacillus paracasei*’. LWT Food Science Technology, 38, pp. 173–180.

Codex Alimentarius Comitte. (2011) *Codex Standard For Fermented Milk*. Codex Stan 243. Roma: Food and Agriculture Organization

Collado, M. C. M., Gueimonde., and Salminen, S. (2010) ‘Probiotics in adhesion of pathogens: Mechanisms of action’ in: Watson, R. R., and Victor, R. F. (eds.) *Bioactive Food in Promoting Health: Probiotics and Prebiotics*. London: Elsevier

Crowley, S. V., Kelly, A. L., Schuck, P., Jeantet, R., and Mahony, J. A. (2016) ‘Rehydration and solubility chracteristics of high-proteins dairy foods’ in McSweeney, P. L. H., and O'Mahony, J. A. (eds.) *Advanced Dairy Chemistry*. New York: Springer, pp. 99-131

De Simone, C. (2019) ‘The unregulated probiotic market’, *Clinical Gastroenterology and Hepatolology*, 17, pp. 809–817

Dutra, R. M., Gennari, A., Volpato, G., and Volken de Souza, C. F. (2015) ‘Lactose hydrolysis in milk and dairy whey using microbial ßgalactosidases’, *Enzyme Resources*, 2015, pp. 1-7

Filannino, P., Cardinali, G., Rizello, C. G., Buchin, S., De Angelis, M., Gobetti, M and Di Cagno, R. (2014) ‘Metabolic responses of *Lactobacillus plantarum* strains during fermentation and storage of vegetable and fruit juices’, *Applied and Environmental Microbiology*, 80(7), pp. 2206-2215

Gallina, D. A., Barbosa, P. D. P. M., Ormenese, R. D. C. S. C., and Garcia, A. D. O. (2019) ‘Development and characterization of probiotic fermented smoothie beverage’, *Revista Cienca Agronomica*, 5 (3), pp. 378-386

Hadiwiyoto, S. (1994) *Teori dan Prosedur: Pengujian Mutu Susu dan Hasil Olahannya. Edisi Kedua* (*Theory and Procedure: Testing the Quality of Milk and Processed Products. Second Edition*). Yogyakarta: Liberty [In Indonesian]

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B. D. F., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S. J., Calder, P. C., and Sanders, M.E. (2014) ‘Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic’, *Nature Reviews Gastroenterology and Hepatology*, 11 (8), pp. 506-514

Karimi, R., Mohammad, H. A., Mehran, G., and Moharam, V. (2015) ‘Application of inulin in cheese as prebiotic, fat replacer and texturizer: A review’, *Carbohydrate Polymers*, 119, pp. 85–100

Kennedy, D. O. (2016) ‘B vitamins and the brain: Mechanisms, dose, and e_cacy—A review’, *Nutrients*, 8, pp. 68-78

Kycia, K., Chlebowska-´Smigiel, A., Szydłowska, A., Sokół, E., Ziarno, M., and Gniewosz, M. (2020) ‘Pullulan as a potential enhancer of *Lactobacillus* and *Bifidobacterium* viability in synbiotic low fat yoghurt and its sensory quality’, *LWT Food Science Technology*, 128, pp. 1-8

Lahteinen, T., Malinen, E., Koort, J. M. K., Mertaniemi-Hannus, U., Hankimo, T., Karikoski, N., Pakkanen, S., Laine, H. Sinlapaa, H., Soderholm, H., and Palva, A. (2010) ‘Probiotic properties of *Lactobacillus* isolates originating from porcine intestine and feces’, *Anaerobe*, 16, pp. 293-300

Lebeer, S., Peter, A. B., Maria, L. M., Jan-Peter, V. P., Mary, O. M., Collin, H., Bruno, P., Stefan, R., and Todd, K. (2018) ‘Identification of probiotic effector molecules: Present state and future perspectives’, *Current Opinion in Biotechnology*, 49, pp. 217-223

Lengkey, H. A. W., Jan, A. S., and Roostita, L. B. (2013) ‘The effect of various starter dosages on kefir quality’, *Lucrări Ştiinţifice-Seria Zootehnie*, 59, pp. 113-116

Li, S., Aiqian, Y., and Harjinder, S. (2021) ‘Effects of seasonal variations on the quality of set yogurt, stirred yogurt, and Greek-style yogurt’, *Journal of Dairy Science*, 104, pp. 1424-1432

Liptakova, D, Matejcekova, Z and Valik, L. (2017) ‘Lactic acid bacteria and fermentation of cereals and pseudocereals’, *Fermentation Process*, 10, pp. 65459-65465

Liu, Y., Dat, Q. T., and Marc, R. (2018) ‘Probiotics in disease prevention and treatment’, *Journal of Clinical Pharmacology*, 58(10), pp. 164-179.

Luz, C., Jorge, C., Juan, M. Q., Raquel, T., Maximo, V., Maria, G., Jordi, M., and Giuseppe, M. (2021) ‘Probiotic characterization of *Lactobacillus* strains isolated from breast milk and employment for the elaboration of a fermented milk product’, *Journal of Functional Food*, 84, pp. 261-270

Marangoni, F., Pellegrino, L., Verduci, E., Ghiselli, A., Bernabei, R., Calvani, R., Cetin, I., Giampietro, M., Perticone, F., and Piretta, L. (2019) ‘Cow’s milk consumption and health: A health professional’s guide’, *Journal of The American College of Nutrition*, 38, pp. 197–208

Mengesha, Y., Alemu, T., and Belay, T. (2022) ‘A review on factors influencing the fermentation process of teff (*Eragrostis teff*) and other cereal-based ethiopian injera’, *International Journal of Food Science*, 2022, pp. 1-10

Mills, S., Mariela, S., Carmel, G., Paula, M. O., Gwenda, S., Chris, B., Colin, H., Paul, R., and Wilco, C. M. (2011) ‘Inhibitory activity of *Lactobacillus plantarum* LMG P-26358 against *Listeria innocua* when used as an adjunct starter in the manufacture of cheese’, *Microbial Cell Factories*, 10(1), pp. 1-7

Motevaseli, E., Dianatpour, A., and Ghafouri-Fard, S. (2017) ‘The role of probiotics in cancer treatment: Emphasis on their in vivo and in vitro anti-metastatic effects’, *International Journal of Molecular and Cellular Medicine*, 6 (2), pp. 66-76

Narzary, Y., Brahma, J., Brahma, C., and Das, S. (2016) ‘A study on indigenous fermented foods and beverages of Kokrajhar, Assam, India’, *Journal of Ethnic Foods*, 3, pp. 284–291

Nurgrahadi., Ni Nyoman, P., and I Made, S. (2020) ‘Pengaruh perlakuan 3 jenis bakteri asam laktat dan kombinasinya terhadap karakteristik keju kedelai (The effect of treatment of 3 types of lactic acid bacteria and their combinations on the characteristics of soy cheese)’, *Jurnal Ilmu dan Teknologi Pangan,* 9 (4), pp. 412-425 [In Indonesian]

Nuraida, L., Winarti, S., Hana and Prangdimurti, E. (2011) ’Evaluasi in vitro terhadap kemampuan bakteri asam laktat asal air susu ibu untuk mengasimilasi kolesterol dan mendekonjugasi garam empedu (In vitro evaluation of the ability of lactic acid bacteria from breast milk to assimilate cholesterol and deconjugate bile salts)’, *Jurnal Teknologi dan Industri Pangan*, 22(1), pp. 46-52 [In Indonesian]

Pereira, A. L., and Rodrigues, S. (2018) *Fruit Juices Extraction, Composition, Quality and Analysis: Turning Fruit Juice into Probiotic Beverages*. Academic Press. 279-287.

Rahmawati, I. S., Zubaida, E., and Saparianti, E. (2015) ’Evaluasi pertumbuhan isolat probiotik (*L. casei* dan *L. plantarum*) dalam medium fermentasi berbasis ubi jalar (*Ipomoea batatas* L.) selama proses fermentasi (Kajian jenis isolat dan jenis tepung ubi jalar) (Evaluation of the growth of probiotic isolates (*L. casei and L. plantarum*) in sweet potato-based fermentation medium (*Ipomoea batatas L.*) during the fermentation process (Study on types of isolates and types of sweet potato flour)’, *Jurnal Aplikasi Teknologi Pangan*, 4(4), pp. 133-141 [In Indonesian]

Rai, V. R., and Bai, J. A. (2015) *Benefical Microbes in Fermented and Function Food*. London: CFC Press

Rizal, M. S., Enny, S., and Suprihana, S. (2016) ‘Pengaruh waktu dan suhu sterilisasi terhadap susu sapi rasa coklat (Effect of sterilization time and temperature on chocolate flavored cow's milk)’, *Jurnal Ilmu-Ilmu Pertanian “Agrika”*, 10(1), pp. 20-30 [In Indonesian]

Rizzoli, R., and Biver, E. (2018) ‘Effects of fermented milk products on bone’, *Calcified Tissue International*, 102(4), pp. 489–500

Rosiana, E., and Armansyah, T. (2013) ‘Kadar asam laktat dan derajat asam kefir susu kambing yang di fermentasi dengan penambahan gula dan lama inkubasi yang berbeda (Lactic acid content and acid degree of fermented goat's milk kefir with the addition of sugar and different incubation times)’, *Jurnal Medika Veterinaria*, 7(2), pp. 87–90 [In Indonesian]

Sanlier, N., Gokcen, B. B., and Sezgin, A.C. (2019) ‘Health benefits of fermented foods’, *Critical Review Food Science and Nutrition*, 59(3), pp. 506–527

Sarwono, J. (2009) *Statistik Itu Mudah* (*Statistics is Easy*). Yogyakarta: Penerbit Andi [In Indonesian]

Sawitri, M. E. (2011) ‘Kajian konsentrasi kefir grain dan lama simpan dalam refrigerator terhadap kualitas kimiawi kefir rendah lemak (Study of kefir grain concentration and storage time in the refrigerator on the chemical quality of low-fat kefir)’, *Jurnal Ilmu-ilmu Peternakan*, 21(1), pp. 23-28 [In Indonesian]

Savaiano, D. A., and Robert, W. H. (2021) ‘Yogurt, cultured fermented milk, and health: A systematic review’, *Nutrition Reviews*, 79(5), pp. 599-614

Serrano-Nino J. C., Solis-Pacheco, J. R., Gutierrez-Padilla, J. A., Cobian-Garcia, A., Cavazos-Garduno, A., Gonzalez-Reynoso, O., and Aguilar-Uscanga B. R. (2016) ‘Isolation and identification of lactic acid bacteria from human milk with potential probiotic role’, *Journal of Food and Nutrition Research*, 4(3), pp. 170-177

Standar Nasional Indonesia (SNI) (2009). *SNI 2981:2009. Yogurt*. Jakarta: Badan Standardisasi Nasional (BSN) [In Indonesian]

Sudarmadji, S., Haryono, B. dan Suhardi. (1997) *Prosedur Analisa Untuk Bahan Makanan dan Pertanian* (*Analysis Procedures for Foodstuffs and Agriculture*). Bandung: Penerbit Angkasa [In Indonesian]

Suharto, E. L. S., Kurnia, Y. F., and Purwati, E. (2021) ’Total bakteri asam laktat, *total plate count*, dan total asam tertitrasi pada susu kambing fermentasi dengan penambahan sari wortel selama penyimpanan dingin ('Total lactic acid bacteria, total plate count, and total titrated acid in fermented goat's milk with the addition of carrot juice during cold storage)’, *Jurnal Peternakan Indonesia*, 23(2), pp. 102-107 [In Indonesian]

Suharyono, A. S., and Muhamad, K. (2011) ‘Pengaruh konsentrasi starter *Streptococcus thermophillus* dan lama fermentasi terhadap karakteristik minuman laktat dari bengkuang (*Pachyrrhizus erosus*) (Effect of *Streptococcus thermophillus* starter concentration and fermentation time on the characteristics of lactic drinks from Jicama (*Pachyrrhizus erosus*)’, *Jurnal Teknologi Hasil Pertanian*, 3(1), pp. 51-58 [In Indonesian]

Terefe, S. N., and Augustin, M. A. (2020) ‘Fermentation for tailoring the technological and health related functionality of food products’,*Critical Review Food Science and Nutrition*, 60, pp. 2887–2913

Setiarto, R. H. B., and Widhyastuti, N. (2016) ‘Pengaruh fermentasi bakteri asam laktat *Lactobacillus plantarum* B307 terhadap kadar proksimat dan amilografi tepung taka modifikasi (*Tacca leontopetaloides*) (Effect of fermentation by lactic acid bacteria *Lactobacillus plantarum* B307 on proximate levels and amylographies of modified taka flour (*Tacca leontopetaloides*))’, *Jurnal Ilmu Pertanian Indonesia (JIPI)*, 21(1), pp. 7-17 [In Indonesian]

Triyono, A. (2010) ‘Mempelajari pengaruh maltodekstrin dan susu skim terhadap karakteristik yoghurt kacang hijau (*Phaseolus Radiatuus L.*) (Studying the effect of maltodextrin and skim milk on the characteristics of green bean yogurt (*Phaseolus Radiatuus L.*))*’, Seminar Rekayasa Kimia dan Proses*, pp. 4-5 [In Indonesian]

Utami, R., Andriani, M. A. M., and Zoraya, A. P. (2010) ’Kinetika fermentasi yoghurt yang diperkaya ubi jalar (*Ipomea batatas*) (Kinetics of sweet potato enriched yogurt (*Ipomea batatas*) fermentation*’, Journal of Sustainable Agriculture “CarakaTani*”, 25(1), pp. 50-55 [In Indonesian]

Urbaya, L. N., Nazaruddin and Werdiningsih, W. (2017) ‘Pengaruh konsentrasi *Lactobacillus plantarum* terhadap mutu tepung gadung (*Discorea hispida Dennst*) (Effect of concentration of Lactobacillus plantarum on the quality of gadung flour (*Discorea hispida*

Dennst)', *Jurnal Ilmu dan Teknologi Pangan*, 1(1), pp. 1-13 [In Indonesian]

Wahyu, S. S., Julaikha, S., Suranto, S., Susanti, Y., Lorentina, Y., Sopian and Arfah, Z. Y. (2012) *Pengujian Total Bakteri/Total Plate Count (TPC)* (*Testing for Total Bacteria/Total Plate Count (TPC)*. Jakarta: Departemen Pendidikan Nasional [In Indonesian]

Widodo., Tiyas, T. T., Eti, A., Asmarani, K., and Widya, A. (2012) 'Human origin *Lactobacillus casei* isolated from Indonesian infants demonstrating potential characteristics as probiotics *in vitro*', *Indonesian Journal of Biotechnology*, 17(1), pp. 79-89

Wu, H., Tim-Fat, S., and Jiachi, C. (2021) 'Characterization of the probiotic potential of lactic acid bacteria isolated from kimchi, yogurt, and baby feces in Hong Kong and their performance in soymilk fermentation', *Microorganism*, 9(12), pp. 2544-2551

Xiang, H., Sun-Waterhouse, D., Waterhouse, G. I., Cui, C., and Ruan, Z. (2019) 'Fermentation-enabled wellness foods: A fresh perspective', *Food Science and Human Wellness*, 8(3), pp. 203–243

Yunus Y., and Zubaidah, E. (2015) 'Pengaruh konsentrasi sukrosa dan lama fermentasi terhadap viabilitas *L. casei* selama penyimpanan beku velva pisang Ambon (The effect of sucrose concentration and fermentation time on the viability of L. casei during frozen storage of Ambon banana velva)', *Jurnal Pangan dan Agroindustri*, 3(2), pp. 303-312 [In Indonesian]

Zein, S., Yasyifa., Ghozi, R., Harahap, E., Badruzzaman, F.H., and Darmawan, D. (2019) 'Pengolahan dan analisis data kuantitatif menggunakan aplikasi SPSS (Processing and analysis of quantitative data using the SPSS application)', *JTEP-Jurnal Teknologi Pendidikan dan Pembelajaran*, 4(1), pp. 839–845 [In Indonesian]

Zhang, B., Wang, Y., Tan, Z., Li, Z., Jiao, Z., and Huang, Q. (2016) 'Screening of proboitic activities of *Lactobacilli* strains isolated form tradisional Tobatan Qula, a raw yak milk cheese', *Journal of Animal Science*, 29(10), pp. 1490-1499

Zheng, J., Wittouck, S., Salvetti, E., Franz, C., Harris, H., Mattarelli, P. (2020) 'A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus *Lactobacillus* beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*', *International Journal of System Evolution. Microbiology*, 70, pp. 2782–2858