Innovation in Making Buffalo (*Bubalus bubalis*) Milk Probiotic: Dadih

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Abstract: Dadih is a traditional, sustainable food made from fermented buffalo milk, passed down from generation to generation on the island of Sumatera for thousands of years. The COVID-19 outbreak has significantly increased demand for buffalo milk, including fermented buffalo milk/dadih in Sumatera due to its nutritional content. Until now, milk fermentation has been carried out using *Bambusa verticillata* bamboo tube containers. This fermentation process has varied consequences depending on the conditions of each bamboo. Furthermore, bamboo cannot be reused in making dadih, so efforts are underway to find alternative containers for producing dadih, including plastic containers with the addition of a starter culture. This study aimed to identify the optimal conditions for manufacturing dadih in plastic containers using bamboo dadih as the initial culture. The study utilised a factorial completely randomized design comprising two factors. The first factor was the fermentation period, which was 24 to 48 hours. The second factor comprised initial concentrations of 1.5%, 3%, 4.5%, and 6%.

At the beginning of the study, dadih was isolated to obtain a dominant culture, which was then purified and identified using the PCR 16S rRNA method-the study analysed chemical quality parameters, including pH, protein content, and total acid. In addition, it evaluated the biological condition by quantifying the overall number of bacterial colonies. The organoleptic characteristics were assessed based on aroma, color, texture, and taste. The PCR results showed that the dominant bacteria *were Weissella cibaria.* The pH levels across all treatments ranged from 4.53 to 4.62.

Furthermore, the protein level decreased as the proportion of starter and the length of fermentation increased. In contrast, the overall acidity level rose as the proportion of starter and duration of fermentation increased. There was an interaction between the fermentation period and dadih concentration. The organoleptic test showed significant differences in texture and taste due to the treatment using a 3% starter and a fermentation time of 24 hours. The study results suggest that fermenting dadih in plastic containers with a 3% starter for 24 hours produces the most desirable organoleptic effects. The colony count of $2.4x10^8$, which is dominantly by *Weissela cibaria,* indicates that the drink is classified as probiotic.

Keywords: Bamboo, buffalo milk, dadih, fermentation, probiotic.

INTRODUCTION

Buffalo is the mainstay livestock of Asian nations, contributing both meat and milk [1]. The buffalo farming sector has experienced substantial expansion in response to the COVID-19 outbreak. Buffalo milk contains high-quality nutrients and benefits that strengthen the human body's immune system [2]. The need for buffalo milk persists. Processing buffalo's milk to add probiotics might enhance its beverage quality [3]. An alternative strategy is to utilise the fermentation process. For generations, a traditional fermentation method has been employed in Sumatera, utilising bamboo tubes. The milk product fermented in bamboo tubes is widely known as dadih among the local populace.

Dadih contains a high concentration of lactic acid bacteria (LAB) with probiotic properties. Probiotics are living bacteria that attach to the lining of the intestines and have beneficial effects on the health and energy of the host [4]. Probiotics have a significant influence on health through the production of metabolites. These metabolites have various beneficial effects, such as blocking pathogenic bacteria, reducing cholesterol levels, acting as antimutagenic, anticarcinogenic, and anti-vaginitis agents, enhancing the immune system, preventing constipation, and generating B vitamins and bacteriocins [5-7].

In making dadih, bamboo tubes facilitate spontaneous fermentation. The widely used bamboo is typically gombong bamboo (*Bambusa verticillata*) or ampel bamboo (*Bambusa vulgaris*), both of which flourish in different areas. The authors [7, 8] observed that bamboo walls and segments harbor microorganisms that enable fermentation, resulting in dadih. Dadih is rich in a variety of lactic acid bacteria. The identified lactic acid bacteria include *Lactobacillus*, *Pediococcus* [9], *Lactobacillus plantarum* IS-10506 and IS-20506, and *Enterococcus faecium* IS-27526, IS-23427, and IS-16183 [10], as well as Weissella *paramesenteroides* strain JCM 9890 [11].

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The conversion of milk into dadih takes 24 to 48 hours. However, the uniformity of dadih's quality was found to be variable. The quality of dadih depends on factors such as the specific type of bamboo used and the combination of mature and juvenile bamboo. The variation can be attributed to the higher water content in juvenile bamboo. Moreover, the abundance of microorganisms in young bamboo is inadequate.

The production of dadih in bamboo tubes is not environmentally sustainable. Bamboo is a non-renewable material that cannot be recycled or repurposed. Moreover, transporting bamboo tube dadih is hindered by its substantial weight and large dimensions. Therefore, there is a need for an alternative container for dadih production, namely a reusable plastic container. Bamboo dadih is solely used as a fermentation starter. Choosing a starter culture from bamboo dadih would be more practical, as it eliminates the need for a time-consuming isolation procedure.

This study used a plastic container as an alternative to bamboo tubes. Another advantage of using plastic is that it can be used multiple times. It also provides anaerobic conditions, unlike bamboo, which has a porous structure. Bamboo contains flavonoids, which can affect the taste of dadih, while plastic is free of these substances.

To boost the quality of dadih, it is crucial to conduct a study to improve its overall quality. This study aims to determine whether using plastic containers improves the chemical (pH, Protein, Total acids) and biological quality of dadih (LAB colonies). Furthermore, organoleptically, dadih fermented in plastic will have a better aroma, color, texture, and taste.

MATERIALS AND METHODS

The study was conducted at the Animal Production Laboratory of the Animal Husbandry Study Program, Universitas Sumatera Utara.

MATERIALS

Buffalo milk, bamboo tubes, 300 ml plastic containers, MRS Agar media (de Man, Rogosa, and Sharpe Agar), a pH meter, and Petri dishes were among the materials used in this investigation. Buffalo milk was purchased from a buffalo milking farm. Every 6 a.m., the milking process begins. Before milking, the farmer cleans the buffaloes with water, then wipes the teats with warm water. Buffalo teats are milked into clean containers, which are then transferred to the milk storage room when full. The milk is then filtered through a clean sieve and packaged in 1-liter plastic bags for sale. Purchased milk is immediately taken to the laboratory and pasteurized.

METHODS

The study commenced by fermenting buffalo milk in bamboo tubes (*Bambusa verticillata*) to produce dadih (Figure **2**). After a 48-hour fermentation period, the dadih is extracted from the bamboo and blended to achieve uniform consistency between its solid and liquid components. Subsequently, this dadih serves as a culture to initiate the production of the subsequent dadih, which is made in a plastic container. Dadih was examined in the Animal Production Laboratory to determine pH, protein content (%), total acid, and LAB bacterial population (Table **2**). Isolation of dadih was conducted to obtain the dominant culture, which was further investigated to determine its species.

Isolated Dominant Culture

In this study, the dominant culture from the dadih was isolated. A 1-gram dadih sample was placed in an Erlenmeyer flask containing 9 mL of sterile MRS broth and incubated on a shaker at 37 °C for 48 hours. Dilution was performed by adding 1 mL of culture medium to an Erlenmeyer flask containing 9 mL of sterile MRS broth. The diluted liquid culture medium was inoculated onto MRS agar using the spread method, and the plate was incubated for 48 hours at 37 °C. Prominent lactic acid bacteria colonies in MRS agar media were selected and re-inoculated into MRS agar media using the streak method and incubated for 48 hours at 37 °C to obtain pure lactic acid bacteria colonies. Next, this pure LAB was molecularly identified using the 16S rRNA gene by Indolab Singapore via the Polymerase Chain Reaction (PCR) technique (Figure **1**).

A plastic container was filled with 150 ml of buffalo milk, to which the starter was then added as treatments, followed by fermentation to become dadih (Figure **2**). The temperature of the milk after adding the starter was 30 °C. The method used in this study was an experimental approach using a Factorial Completely Randomized Design (CRD) with a 2x3 factorial pattern, with three replications per unit. Two factors were examined in this study, which are as follows:

Factor 1: Fermentation duration ranged from 24 to 48 hours.

Primer Information

Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
LC096236.1	Weissella cibaria	1516	4	1516	99	2791	0.0	1512/1513	99.93

Kingdom	Family	Genus	Species
Bacteria	Lactobacillaceae	Weissella	Weissella cibaria

Figure 1: 16S rRNA PCR result of dadih dominant culture.

Figure 2: Dadih in bamboo and plastic containers.

D1: 24 hours; D2: 48 hours

Factor 2: Starting concentrations of 1.5%, 3%, 4.5%, and 6%.

C1: 1.5%; C2: 3%; C3: 4.5%, C4: 6%

The data analysis method for factorial RAL is as follows:

Yijk : μ+ αi+ Qj+ ($\alpha\beta$)ij + εijk

Description:

- $Y_{(i\square\square)}$: Observation values in experimental units
- μ: Mean value of observation
- a_i: The influence of factor D (duration)
- β□: The influence of factor F (fermentation)
- $(a\beta)_{i\square}$: Interaction between factor D and factor F at every level
- $\varepsilon_{i\square\square}$: Experimental error for factor D, factor F, in replication k

The treatment combination in this trial was as shown in Table **1**.

The research parameters included chemical quality factors such as pH, protein content, and total acid levels, as well as biological quality factors (i.e., complete colony bacteria) and organoleptic testing.

pH Measurement

The pH of the sample was measured using a digital pH meter (Hanna Instruments) that had been pre-calibrated with buffers of pH 4.0 and pH 7.0, corresponding to the pH range of the dadih. 10 g dadih was weighed and placed in a glass beaker, and distilled water was added to reach a final volume of 10 mL while crushing. The pH electrode was immersed in the dadih medium, and the pH meter was allowed to stabilize to obtain an accurate pH value.

Protein Measurement

Quantitative tests using the Kjeldahl method include the destruction stage, distillation stage, titration stage, and calculation of protein content [12]. Calculation based on the formula :

$$\%\,N = \frac{(V1 - V2)\ x\ N\ x\ 0{,}014\ x\ fp}{W}\ x\ 100$$

%protein=%Nx6,38 factor

Note:

V1=Volume HCL 0,1

V2=Volume HCL 0,1

N for titration of blank solution (ml) N=Normalitas HCL

W=Sample weight (g)

Fp=Dilution factor (5 times)

Table 1: Research the Treatment of Dadih in Plastic Containers Instead of Bamboo

Duration of fermentation - D (in hours)	Dadihconcentration - C (as starter in %)			
Duration of fermentation - D (in hours)	1.5	3	4.5	6
24	D1C1	D1C2	D1C3	D1C4
48	D2C1	D2C2	D2C3	D2C4

Total Acid Measurement

Acidity testing is carried out by titrating lactic acid with 0.1 M NaOH using the AOAC method (2002) [13]. A 10 mL sample is dripped with 2 drops of 1% phenolphthalein (PP) indicator. The sample is then titrated with 0.1 N NaOH until a constant pink color appears. The acid content is calculated using the formula:

$$\text{Total Acid} = \frac{\text{V1 x N x B}}{\text{V2 x 1000}} \text{ x } 100\%$$

V1: Volume of NaOH (mL)

V2: Volume of sample (mL)

N: Normality of NaOH (0.1 N)

B: Molecular weight of lactic acid (90)

Statistical Analysis

Data were analyzed using ANOVA, followed by Duncan's multiple range test ($P<0.05$) in SPSS version 27.0.

Organoleptic Testing

Organoleptic testing was conducted using a hedonic scale that assesses aroma, color, texture, and taste. Thirty semi-trained panelists, selected based on previous sensory experience and a brief orientation training-including an introduction to sensory attributes and hedonic assessment-evaluated the samples. Each dadih sample was tested three times to ensure the validity of the results. The organoleptic scale in this study refers to [14]: 1 (dislike very much), 2 (dislike), 3 (somewhat like), 4 (like), and 5 (like very much).

RESULTS AND DISCUSSION

Result of 16S rRNA PCR of the Dominant Culture

The pure culture obtained from the dadih was investigated using the 16S rRNA PCR method at the Macrogen Singapore lab. found to be of the Weissella genus and *Weissella cibaria* species. Identification in the GenBank database at NCBI (National Center for Biotechnology Information) revealed that Weissella is a genus of Gram-positive bacteria belonging to the family Leuconostocaceae. *Weissella cibaria* has also been described as a probiotic.

Chemical Quality of Dadih

Chemical quality data from this study include pH, protein, and total acid. These data are presented in Table **2**.

Table **2** shows that increasing the number of starters during dadih fermentation results in greater milk breakdown and higher acid levels; this was also observed in the studies by [15, 16]. Extending the fermentation time to 2 days made dadih more acidic due to increased bacterial growth. This led to a more significant breakdown of carbohydrates and the production of a larger quantity of organic acids. A study by [17] revealed that the pH level of buffalo milk dadih is approximately 4.55. The pH fluctuations can be attributed to the presence of lactic acid bacteria, which are responsible for the deterioration of milk. Moreover, the pH of milk is directly linked to its nutritional composition, which, in turn, promotes bacterial proliferation [18].

In this study, the inclusion of a starter and the prolonged fermentation process reduced the pH of the dadih, thereby increasing its acidity. Bacteria perform a thorough milk transformation, which involves modifying their protein content. Lactic acid bacteria rely on the nutrients present in milk to support their growth and spread throughout the fermentation process. As the starter concentration and fermentation time increase, the protein concentration in dadih decreases. There is an interaction between the starter concentration and the fermentation period for growth. During this experiment, the protein concentration declined to 4.25, whereas a prior study [19] documented a protein decrease to 4.65. Milk protein, first synthesised by bacteria as a large molecule, is broken down by catabolism to form small peptides that may be easily digested. This is consistent with the research conducted by [20].

Table 2: Chemical Quality of Dadih

pH

Bamboo Dadih	Replication			Average ± SD
	U1	U2	U3	
Bamboo Dadih	4.75	4.49	4.62	4.62 ± 0.13

Factor 1 (Days)	Factor 2 (Doses)				Average ± SD
	C1	C2	C3	C4	
D1	4.59	4.58	4.57	4.57	4.5775a ± 0.8181
D2	4.56	4.53	4.53	4.53	4.5375a ± 0.17110
Average ± SD	4.5750a ± 0.1372	4.5550a ± 0.1441	4.5500a ± 0.1431	4.5500a± 0.1431	

Description: The same superscript in the same column indicates no significant difference (P>0.05)

Protein

Bamboo Dadih	Replication			Average ± SD
	U1	U2	U3	
Bamboo Dadih	4.83	4.59	4.71	4.71a ± 0.12

Factor 1 (Days)	Factor 2 (Doses)				Average ± SD
	C1	C2	C3	C4	
D1	4.71	4.66	4.64	4.56	4.6025a ± 0.72080
D2	4.33	4.30	4.28	4.25	4.2900a ± 0.08323
Average ± SD	4.495a ± 0.40263	4.47a ± 0.66609	4.42a ± 0.77431	4.4a ± 0.25132	

Description: The same superscript in the same column indicates no significant difference (P>0.05)

Total Acid

Bamboo Dadih	Replication			Average ± SD
	U1	U2	U3	
Bamboo Dadih	0.43	0.31	0.37	0.37a ± 0.06

Factor 1 (Days)	Factor 2 (Doses)				Average ± SD
	C1	C2	C3	C4	
D1	0.37	0.42	0.45	0.46	0.4475a ± 0.02491
D2	0.50	0.51	0.57	0.59	0.5417b ± 0.04745
Average ± SD	0.46a ± 0.05727	0.4783ab ± 0.3312	0.515bc ± 0.06285	0.525c ± 0.07259	

Description: The same superscript in the same column indicates a significant difference (P<0.05).

The pH fluctuations directly affect the overall acidity of the dadih. As the pH falls, the total amount of acid increases. Evaluating titratable acidity provides information on the activity of lactic acid bacteria in breaking down lactose in milk. As the number of bacteria increases, more lactose is broken down, leading to a reduction in pH and an increase in titratable acid production. A study by [21] found that dadih had a total acid concentration range of 0.51 to 1.70, and that total acid concentration varied by buffalo milk origin. Different places used to feed buffalo with different grasses, which impact the pH and acidity of dadih [22].

Based on SPSS Statistics (27.0), fermentation time significantly influences pH, protein content, and total acid in dadih. Fermentation for 48 hours resulted in a more acidic pH and decreased protein content, while total acid also increased.

Biological Quality of Dadih

The data analysed in this study pertains to the total number of bacterial colonies present in the biological quality. Table **3** displays the total number of bacterial colonies (TPC).

The total plate count (TPC) of dadih stored in bamboo containers is lower than that of dadih stored in plastic containers. The porous nature of bamboo hinders the establishment of an ideal anaerobic environment required for the proliferation of lactic acid bacteria. This investigation involves observing the interior of the bamboo. Mature bamboo typically exhibits desiccated inner walls and segments, which affect the starter's condition. This study's total plate content (TPC) of bamboo dadih is 5 × 10^6 CFU/g (6.7018 Log10 CFU/g). The number of colonies is more significant in dadih fermented in plastic tubes. The maximum (TPC) observed in dadih fermented with 6% starter and held in plastic tubes for 2 days is 2.4 x 10^9 CFU/g (9.380211 Log10 CFU/g). A study by [23] investigated fermentation in plastic packaging and reported a total plate count (TPC) of 2.4 x 10^{10} CFU/g (10.38021 Log10 CFU/g). This disparity could be attributed to variations in milk quality or the number of distinct starters used. In this study, the cumulative bacterial count in dadih is 109, thus qualifying at to be called a probiotic drink [24]. According to [25], the most commonly utilised probiotic in dadih is *Lactobacillus*, whereas in this study, it is *Weissella cibaria* from the genus *Weissella*.

Based on SPSS Statistics (27.0), dadih concentration and fermentation time significantly influence TPC. A dadih concentration of 6% and a fermentation time of 2 days resulted in significantly higher TPC than other treatments.

Aroma

The dadih's aroma received a rating of 4 on the hedonic scale, suggesting that the panellists generally found it enjoyable (Table **4**). Panellists significantly prefer the 3% starter treatment and 2 days of fermentation. The panellists describe the aroma of dadih as unique and refreshing, with a sourness. As stated by [26], the delightful, tangy aroma of buffalo milk dadih results from the enzymatic conversion of lactose into diacetyl. The bacteria present in dadih, such as *Leuconostoc and Lactobacilli*, while in this study, is *Weisella cibaria,* which metabolises lactose. This process produces a noticeable diacetyl aroma.

Furthermore, it is essential to note that this aroma will be strong and intense in the early phases of fermentation. The residents of Sumatera possess a profound fondness for this scent. Thus, dadih is specifically made to be the primary complement to rice. Furthermore, they include freshly chopped chillies and onions in the dadih, which complements the accompaniments. This suggests that each panellist has a very similar level of preference and a strong liking for the scent of freshly prepared sour dadih. In this study, *Weissela cibaria* plays a role in imparting a favorable aroma to dadih, as it is the dominant bacterium.

Color

The complete hedonic scale produces a numerical score of 3 (Table **4**), indicating that the white colour of

Table 3: Biological Quality of Dadih (Log10 CFU/g)

Bamboo Dadih	Replication			Average ± SD
	U1	U2	U3	
Bamboo Dadih	6.70757	6.690196	6.70757	6.7018 ± 0.01003

Factor 1 (Days)	Factor 2 (Doses)				Average ± SD
	C1	C2	C3	C4	
D1	8.321891	8.37996	8.486303	8.612698	8.4502a ± 0.11678
D2	8.763385	9.321891	9.342123	9.37996	9.2018b ± 0.26570
Average ± SD	8.5426a ± 0.24222	8.8509b ± 0.51621	8.9142c ± 0.46912	8.9963d ± 0.42046	

Description: The same superscript in the same column indicates a significant difference (P<0.05).

Table 4: Organoleptic Test for Buffalo milk Dadih

Aroma

Bamboo Dadih	Replication			Average ± SD
	U1	U2	U3	
Bamboo Dadih	4.11	4.13	4.09	4.11 ± 0.02

Factor 1 (Days)	Factor 2 (Doses)				Average ± SD
	C1	C2	C3	C4	
D1	4.22	4.21	4.25	4.32	4.25a ± 0.0518
D2	4.21	4.45	4.21	4.21	4.27a ± 0.1132
Average ± SD	4.2150a ± 0.0187	4.33c ± 0.1345	4.23ab ± 0.0389	4.2650b ± 0.0723	

Description: The different superscripts in the same column indicate a significant difference (P<0.05).

Color

Bamboo Dadih	Replication			Average ± SD
	U1	U2	U3	
Bamboo Dadih	3.12	3.14	3.1	3.12 ± 0.02

Factor 1 (Days)	Factor 2 (Doses)				Average ± SD
	C1	C2	C3	C4	
D1	3.15	3.23	3.16	3.22	3.19a ± 0.042
D2	3.22	3.13	3.23	3.13	3.1775a ± 0.0551
Average ± SD	3.1850a ± 0.0423	3.18a ± 0.0583	3.1950a ± 0.0497	3.1750a ± 0.0532	

Description: The same superscript in the same column indicates no significant difference (P>0.05).

Texture

Bamboo Dadih	Replication			Average ± SD
	U1	U2	U3	
Bamboo Dadih	3.11	3.12	3.1	3.11 ± 0.01

Factor 1 (Days)	Factor 2 (Doses)				Average ± SD
	C1	C2	C3	C4	
D1	3.23	4.65	4.25	4.25	4.0950b ± 0.5495
D2	3.24	3.25	3.26	3.22	3.2425a ± 0.0249
Average ± SD	3.235a ± 0.0235	3.95c ± 0.7672	3.755b ± 0.5426	3.735b ± 0.5652	

Description: The different superscripts in the same column indicate a significant difference (P<0.05).

Taste

Bamboo Dadih	Replication			Average ± SD
	U1	U2	U3	
Bamboo Dadih	2.3	2.2	2.4	2.3 ± 0.1

Factor 1 (Days)	Factor 2 (Doses)				Average ± SD
	C1	C2	C3	C4	
D1	4.23	4.6	4.18	4.18	4.2975b ± 0.2247
D2	4.18	3.9	3.16	3.28	3.705a ± 0.5128
Average ± SD	4.205b ± 0.0472	4.4c ± 0.2967	3.67a ± 0.5601	3.73a ± 0.4962	

Description: The different superscripts in the same column indicate a significant difference (P<0.05).

the dadih is somewhat liked among the panellists. Nevertheless, they do not manifest any dislike or inclination. This can be attributable to the panellists' understanding that dadih, made from buffalo's milk, is white. The dadih in this investigation has a consistent, homogeneous white colour across all treatments, as it was made from buffalo's milk and the same starter culture. The white colour of buffalo dadih is ascribed to its vitamin A content [27]. The statistical analysis concluded no significant difference between the panelists. This suggests that each panellist has a virtually identical preference for the colour of traditional buffalo's milk dadih products.

Texture

Panelists state that the treatment of 3% starter addition with 1 day fermentation has the best texture (Figure **3**), which is solid but soft. At the same time, bamboo dadih at 1 day of fermentation and 1.5% starter treatment is not solid. This is possible because bacterial growth is suboptimal, so the organic acids are insufficient to solidify the dadih. However, it differs from the 6% treatment, where the dadih is not solid enough because it is influenced by water produced from the breakdown of milk. This water indicates that milk breakdown has been more active due to the presence of many starters. In general, the 2-day fermentation treatment with the addition of starter showed that the bacteria had worked optimally, as indicated by the formation of a lot of water, with the solid part floating above the liquid. Using a starter from bamboo dadih is effective for making dadih in plastic containers. However, the best results are when the starter is 0 to 7 days old. Bamboo starter that is more than 7 days old, even when stored in the refrigerator, when applied to fermented milk in plastic containers, the dadih solidifies, but the flavor is not sour, and the texture is less soft. Possibly, there is a change in the composition of the hetero bacteria present in the starter. According to [27], after several days, yeast grew by utilizing lactic acid produced by *Lactobacillus,* thereby suppressing Lactobacillus growth. The most prevalent yeast is *Candida metapsilosis*.

Figure 3: Dadih resulted from a 3%starter with a fermentation time of 1 day.

Taste

Panelists expressed a dislike for bamboo dadih and a clear preference for dadih in plastic containers. The organoleptic evaluations were around 2, according to the panelists' observations of the dadih preserve in bamboo tubes, due to a slightly bitter taste. Nevertheless, dadih preserved in plastic tubes does not have a bitter flavor, which is reflected in its score of 4. Bamboo tube dadih, either from bamboo gombong or ampel, is slightly bitter, and the ancients stated that it is favorable because ants do not disturb the dadih [28]. Panellists agreed that dadih in a plastic container with 3% and 1 day fermentation was the most favourable.

The bitterness of dadih is caused by its flavonoids, saponins, resins, fixed oils, phytosterols, phenols, and tannins [29]. Traditionally, bamboo was primarily recognized as a container for fermentation. Inside the bamboo, naturally occurring bacteria help coagulate the milk. Nowadays, many kinds of plastic containers can be used as fermentation containers by adding starter from bamboo dadih. The resulting dadih is better; results can be controlled because the solidified dadih can be identified by its appearance in the container. Using a plastic container will be very helpful for monitoring the dadih solidification process. The characteristics of the starter derived from bamboo tubes are unpredictable due to variances in the age of the bamboo used, which subsequently alters the bacterial condition.

Panelists' preferences became clearer through taste tests of dadih in plastic containers. This provides a strong foundation for the potential commercial development of dadih in plastic containers, using treatments such as those in this study, with *Weissella cibaria* as the dominant culture.

CONCLUSIONS

Fermenting dadih in plastic containers can improve its quality. The panellists preferred a preferred aroma produced from dadih in a plastic container, with a concentration of 3% and a fermentation time of 1 or 2 days, while either texture or taste, panellists preferred dadih with a concentration of 3% and 1 day

fermentation. The dadih flavour displayed a refreshing sour taste, with a pH of 4.5, a protein content of 4.6, and a total acid concentration of 0.54. The dadih exhibited a resilient, velvety consistency and a bacterial count of 2.4 × 10^8, which could be classified as probiotic, with *Weissella cibaria* as the dominant probiotic. The results of this research will be patented and developed for use in the production process.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used for this research are available from the corresponding author upon request.

DECLARATION OF INTEREST

The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

AUTHORS CONTRIBUTION

Nurzainah Ginting conceptualized and conducted the experiments, while Raden Edhy Mirwandhono analyzed the results and wrote the manuscript. Galih Ari Wirawan Siregar, Yuan Yu Lin, and Wahyudi Himawan Sutanto contributed to the conceptualization of the research study, as well as its finalization, review, revision, and proofreading for publication.

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