




Review

Characterization of *Dadih*: Traditional Fermented Buffalo Milk of Minangkabau

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Abstract: *Dadih* or *dadiah* is traditional fermented buffalo milk of Minangkabau, which occurs spontaneously. *Dadih* is commonly served as *ampiang dadih*, or other dishes. The microbiota found in *dadih* are dominated by lactic acid bacteria, and yeasts are also found. The lactic acid bacteria provide functional value, such as antimicrobial, hypocholesterolemic, antimutagenic, antioxidant, and immunomodulatory properties, as well as being the source of γ -aminobutyric acid (GABA) as an anti-stress agent and folate. Nevertheless, many challenges were observed in *dadih* production, including the limitation of buffalo milk production due to decreasing populations of buffalo in the last two decades, unstandardized *dadih* production due to the spontaneous fermentation in natural bamboo tubes, and safety problems as no heat treatment is applied in the production of *dadih*. These problems impede the development of *dadih* production, thus is it important to improve buffalo cultivation through artificial insemination programs, using different types of milk and pasteurization processes in *dadih* production, and incubator development to accelerate the fermentation period.

Keywords: *dadih*; buffalo milk; lactic acid bacteria; fermentation; health value; traditional food



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1. Introduction

Indonesia is a multicultural country with more than 1300 ethnic groups [1]. Each region or ethnic group in Indonesia has unique traditional foods with a different history, purpose, raw materials, and taste. Traditional food can be the symbol or historical evidence that differentiates one ethnic group from another. Minangkabau is one of the well-known ethnic groups in Indonesia. In 2010, the total population of the Minangkabau (Minang) people was 2.73% of the total population in Indonesia, and dominantly resided in West Sumatra province (Figure 1) [1]. Besides *lamang* [2], *lamang tapai* [3], *rendang* [4], and *ketupat bareh* [5], *dadih* is also known as the authentic traditional food originated from this region.

Dadih (Figure 2) is a fermented buffalo milk product in a bamboo tube, which has been prepared and consumed by the Minang people for hundreds of years. It is a yogurt-like product with a smooth and glossy surface, even consistency, creamy color, good aroma, and sour taste. Minang people call it as *dadiah*, and it is easily found in Padang Panjang, Bukittinggi, Lima Puluh Kota, Solok, and Tanah Datar regions [6]. Since *dadih* is produced in home industries, it is not very popular compared to other fermented milk products [7].

Various dishes can be made from *dadih* (Figure 3). One of the popular dishes in Minangkabau made from *dadih* is *ampiang dadih* (Figure 3A). Additionally, *dadih* is also served as a traditional dish at weddings and ethnic ceremonies while giving the title “Datuk” (the title of respect) in West Sumatra [6].



Figure 1. The map of ethnic groups in Sumatra islands and surroundings, Indonesia. The light and dark olive color on the center of the map shows the Minangkabau ethnic group, producing the authentic traditional fermented buffalo milk—*dadih*. Picture by: Gunawan Kartapranata, based on “Peta Suku Bangsa di Indonesia” at National Museum of Indonesia, Jakarta (CC BY-SA 3.0, source: https://upload.wikimedia.org/wikipedia/commons/archive/8/8c/20180810080403%21Sumatra_Ethnic_Groups_Map_en.svg) (accessed on 16 May 2021).



Figure 2. *Dadih*. The bamboo tube is used as the container during buffalo milk fermentation process. The resulting fermented buffalo milk—*dadih* has a smooth and glossy surface, even consistency, creamy-white color, pleasant aroma, and sour taste. Photo by: I. Soemardjan (CC BY-NC-ND 2.0, source: <https://www.flickr.com/photos/94093643@N00/180430205>) (accessed on 16 May 2021) and <https://www.flickr.com/photos/indrani/180430424/in/photostream/>) (accessed on 16 May 2021).



Figure 3. *Dadih*—fermented buffalo milk in various dishes. (A) *Ampiang dadih* (*ampiang dadiah*). Traditional food of Minangkabau, consists of *dadih*, *ampiang* (glutinous rice flakes), shaved ice, shredded coconut or coconut milk, and palm sugar syrup as the topping. Photo by: Dinda (CC BY-NC 2.0, source: <https://www.flickr.com/photos/41912023@N07/8908205779>). (B) *Dadih*—fermented buffalo milk served with sliced shallot and chili. Authors hold the copyright. (C) Beef curry (*rendang*). *Dadih*—fermented buffalo milk can be used as the substitute of coconut milk in beef curry. Photo by: Alpha (CC BY-NC 2.0, source: <https://www.flickr.com/photos/avlxyz/39545761832/in/photostream/>) (accessed on 29 May 2021).

The fermentation in *dadih* production occurs spontaneously, without inoculating of any starter cultures and without heat or pasteurization [8]. As no heat treatment is applied in *dadih* production, *dadih* does not fulfill any standards which require pasteurization of the raw milk [6]. The coagulation of fresh buffalo milk into *dadih* occurs due to the presence of indigenous enzymes and indigenous natural lactic acid bacteria (LAB) in buffalo milk [6]. The indigenous LAB may vary from one place to another and from time to time due to this spontaneous fermentation [6,7]. Despite good hygiene practice not being implemented in the production process of *dadih*, the natural LAB found in *dadih* contributes to the safety of this product [6]. However, the standardization of the production process is needed to ensure safety and to maintain the properties of *dadih*.

Dadih is known as a potential probiotic as it has a huge amount of LAB (typically 10^8 colony-forming units (cfu/g) [8,9], which potentially support the intestinal wall and has many benefits for life and health of the host [10]. These LAB of *dadih* were reported to have antimicrobial (antipathogenic) properties [10], hypocholesterolemic properties [11–13], antimutagenicity properties [14], antioxidant properties [15], immunomodulatory properties [16,17], anti-stress properties [18,19], and folate-producing ability [20].

Nevertheless, many challenges were observed in *dadih* production which triggers the local government and many researchers to contribute in finding the best solution. Therefore, this article aims to generate and spread the knowledge about the story behind *dadih* in Minangkabau, processing of traditional *dadih*, maturation of *dadih*, microbiota found in *dadih*, nutritional value and chemical analysis of *dadih*, functional value, *dadih* in daily diet, challenges, and future trends of *dadih*.

2. Buffalo, Minangkabau, and *Dadih*

For centuries, the water buffalo (*Bubalus bubalis*, the domestic buffalo) was an important animal to the Minang people. The buffaloes were used by local people for transportation, delivering goods, plowing fields, milling sugar cane in sugar production, and for tourism purposes, such as buffalo fighting or *adu kerbau* [7]. Furthermore, the roof of the traditional Minangkabau house, also known as *Rumah Gadang*, resembles the horns of the buffalo and this also symbolizes the victory of the buffalo of Minangkabau against the Javanese buffalo in the territorial dispute [21].

The products from buffalo itself were also consumed, for example, as a source of meat, milk (including *dadih* production), skin crackers, while the excrement of buffalo is used for

organic fertilizer and biogas production. The production of *dadih* from buffalo milk can help the economy and support the food security of the Minang people [7]. Besides that, for the Minang people, *dadih* also symbolizes the expression of the family host's feelings towards their guests [22].

3. Processing of Traditional *Dadih*

The main materials used in *dadih* production are buffalo milk, bamboo tubes, and banana leaves. The buffalo milk generally contains higher total solids content compared to other mammals' milk (Table 1), especially due to the higher casein and fat content, which are responsible for the creamy, thick body, and custard-like consistency of *dadih* [6]. The higher fat content of the milk results in softer texture and developed flavor of *dadih* [6,11].

Table 1. Comparison of mammals' milk composition.

Mammals	Percent Composition (% <i>w/v</i>) of Mammals' Milk						Reference
	Fat	Casein	Whey Protein	Lactose	Ash	Total Solid	
Buffalo	7.4	3.2	0.6	4.8	0.8	17.2	[6,23]
Cow	3.7	2.8	0.6	4.8	0.7	12.7	[6,23]
Goat	4.5	2.5	0.4	4.1	0.8	13.2	[6,23]
Sheep	7.4	4.6	0.9	4.8	1.0	19.3	[6,23]
Mare	1.9	1.3	1.2	6.2	0.5	11.2	[6,23]
Camel	5.4	2.9	1.0	5.1	0.7	15.0	[24]
Sow	6.8	2.8	2.0	5.5	ND	18.8	[6,23]

ND: Not determined; *w/v*—weight/volume.

Bamboo tubes are chosen as the containers during fermentation because they have hygroscopic properties to keep the product from whey separation, and the bitter taste of bamboo can prevent contamination against ants [6,7]. Many types of bamboo used in *dadih* fermentation were reported, including bamboo ater (*Gigantochloa atter*), bamboo gombong (*Gigantochloa verticillata*), bamboo lengka tali (*Gigantochloa hasskarliana*), bamboo ampel (*Bambusa vulgaris*), and bamboo betung (*Dendrocalamus asper*) [6,7,25]. Among these bamboo types, the native Minang people prefer to use bamboo gombong [6]. The number of indigenous microbes found on the inner surface of bamboo was 2.5×10^2 – 1.0×10^3 cfu/cm², and acid-producing proteolytic bacteria were found to be in the majority [26], followed by yeast [25]. The type of bamboo also affects the flavor of the resulting *dadih* [7]. By analyzing the flavor, color, aroma, and texture in hedonic evaluation, the salad dressing from goat milk *dadih* fermented in bamboo ampel was significantly preferred ($p < 0.05$) compared to that in bamboo gombong, especially within 2 days of the fermentation process [25].

Banana leaves are commonly used to cover the bamboo tube during *dadih* fermentation. The purpose of using banana leaves is to create the facultative anaerobic condition which is optimum for the fermentation process, as well as to avoid unwanted contamination [27]. However, depending on the region, not only banana leaves, but also taro leaves or plastics are used to cover the bamboo [28]. To tie the cover, banana bark or rubber bands are used [27].

The traditional production process of *dadih* is shown in Figure 4. In West Sumatra, most buffaloes produce around 2 L of milk a day [7]. After milking the buffalo, the raw buffalo milk (free from antibiotics) is directly filtered to reduce the physical contamination such as stones and grass. The filtered milk is then poured into the bamboo tube, covered with banana leaves, and tied with a rubber band or banana bark. The fermentation process occurs spontaneously at room temperature (28–30 °C) for 24 h [6] or even 2–3 days [11,13]. The indigenous LAB and proteases will perform the lactic acid fermentation as well as proteolytic activity to produce the *dadih* with a thick consistency, solid texture, smooth surface, and pleasant flavor. The fermentation by LAB is very important to reduce the contaminant by pathogens and spoiling bacteria, thus makes *dadih* safer to consume. The possible mechanisms are (1) by producing the inhibitory metabolites, for example, the organic acids (mainly lactic acid) during the fermentation process, which then decreases

the pH of the medium and pathogens cannot grow well [6]; (2) by producing bacteriocins (see Section 7.1).

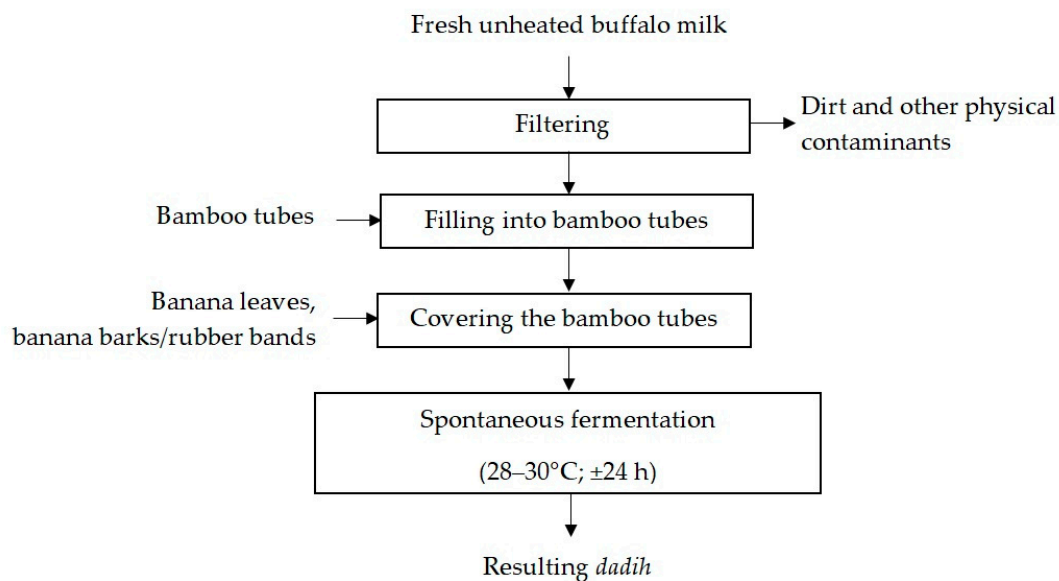


Figure 4. Traditional production process of *dadih*—fermented buffalo milk. Authors hold the copyright.

Dadih is similar to *dahi* from India [8,29], however, they have some differences. In *dadih* production, heat treatment is not applied to the raw buffalo milk, and no starter culture is added for fermentation. While in *dahi* production, the pasteurization process of raw buffalo or cow milk or a combination of both kinds of milk is applied before fermentation, and the back-slopping method (a small quantity of *dahi* from the previous lot is used as a starter culture of the next lot) is used [29,30]. In comparison to yogurt, the fermentation time of *dadih* is longer (24 h up to 3 d) than yogurt (4 h). Furthermore, the mesophilic LAB is involved at 28–30 °C in *dadih* fermentation, while thermophilic LAB is involved at 45 °C in yogurt fermentation [6,29]. The involvement of indigenous yeasts in *dadih* fermentation also differs the *dadih* from yogurt [31].

4. Maturation of *Dadih*

Carbohydrate fermentation, proteolytic activity, and lipid metabolism contribute to the formation of the desired structure and flavor of *dadih*. In carbohydrate catabolism, LAB converts lactose into lactic acid by involving β -galactosidase and lactic acid fermentation. The resulting lactic acid decreases the pH value of buffalo milk and contributes to protein precipitation or coagulation of buffalo milk, so the curd or *dadih* is formed. Decreasing pH value from 6.92 (raw buffalo milk) to 4.65 after 2 days of *dadih* fermentation was observed [32]. Through carbohydrate catabolism, small quantities of flavor compounds are also formed through the volatile fatty acids, ethanol, acetoin, acetic acid, butanone, diacetyl, and acetaldehyde [29]. Lactic acid, as the major fermentation product, is produced by the homofermentative LAB, such as *Lactobacilli*, *Lactococci*, *Pediococci*, and *Streptococci*, while flavor formation is formed by heterofermentative LAB such as *Levilactobacillus brevis*, *Limosilactobacillus fermentum*, and *Leuconostoc* sp. [29]. Lactic acid formed by LAB contributes to the destabilization of casein micelles to form curd and gives the distinctive and characteristic sharp acidic taste of *dadih* [29]. Moreover, *dadih* is safely consumed by people with lactose intolerance [33], as during *dadih* fermentation, the β -galactosidase activity and lactic acid fermentation can help the digestion of lactose.

The proteolytic system of LAB is complicated, especially in turning milk casein into free amino acids and peptides which are needed for LAB growth. However, the overall proteolytic system of LAB is very weak. To support LAB growth, raw buffalo milk possesses

indigenous enzymes that may also contribute to making amino acids and peptides. Since heat is not applied during *dadih* production, the thermal breakdown of raw buffalo milk protein, denaturation, and coagulation of albumins and globulins will not occur. So, the degradation of protein will be catalyzed by bacterial or native proteases. The proteolytic activity from milk proteases, such as plasmin or proteases of bacterial origin, help in the thickening, gelation, and coagulation process of buffalo milk in *dadih* production [6].

The natural lipases in the raw buffalo milk may take part in lipid metabolism, while the insignificant amount of free fatty acids and volatile fatty acids are released by microbial contaminants of the buffalo milk [6]. The high-fat content of buffalo milk (twice as high as cow milk) (Table 1) contributes to the creamy textures and various flavors in *dadih* [6].

5. Microbiota of *Dadih*

In spontaneously fermented products, various types of microbiotas might be involved, including, but not limited to, LAB and yeasts. In *dadih*, the indigenous LAB of buffalo milk are the main group of microbes that are involved in *dadih* fermentation. The LAB will dominate and grow during the fermentation, decrease the pathogenic and spoilage bacteria, and develop the flavor of *dadih*. The total viable LAB count was in the range of 1.42×10^8 – 3.80×10^8 cfu/g [34]. Various types of LAB can be found from one place to another in West Sumatra [35]. *Leuconostoc paramesenteroides*, which produce acetic acid, diacetyl, and other volatiles as the aromatic compounds were reported as the predominant strains of LAB [36]. Nuraida [37] reported that *Leuconostoc mesenteroides*, *Lactococcus lactis* subsp. *lactis*, *Levilactobacillus brevis*, *Lacticaseibacillus casei*, *Lactiplantibacillus plantarum* subsp. *plantarum*, *Enterococcus faecium*, *Limosilactobacillus fermentum*, and *Lacticaseibacillus rhamnosus* were found in *dadih* from buffalo milk. The unhygienic process applied in *dadih* production becomes the possible reason, as *Enterococci* group bacteria, such as *Enterococcus faecalis* was also found in *dadih* [29,38]. Furthermore, the types of fermentation (spontaneous and back-slopping) affect the diversity of LAB in *dadih*, as back-slopping fermentation results in greater diversity compared to spontaneous *dadih* fermentation [39]. Bifidobacterium (18% of total microbiota composition) was found in non-pasteurized *dadih* from the Palupuh region, prepared using back-slopping method [40]. The various types of LAB in *dadih* are mentioned in detail in Table 2.

Not only LAB, but other types of microbes were also found in *dadih*. A higher abundance of species of the order *Serratia* and *Burkholderiales*, and a lower abundance of *Brachybacterium* were found in *dadih* with the back-slopping method [40]. Three yeast species, namely *Saccharomyces cerevisiae*, *Candida metapsilosis*, and *Kluyveromyces marxianus* were found in *dadih* with *C. metapsilosis* as the most predominant species [32]. The different types of yeast in *dadih*, such as *Candida stelimalicola* and *Pichia jadinii*, were also reported [41]. After 2 days of fermentation, the total viable yeast count was 6.85 log cfu/g, or about 7.08×10^6 cfu/g in *dadih* from the Solok region [30].

Candida sp., including *C. metapsilosis*, are normally found in the gastrointestinal tract, but they are also known as opportunistic pathogens which may be considered as the cause of disseminated candidiasis [42]. *C. metapsilosis* was isolated in the inner part of bamboo tubes of *dadih* [32]. A study reported that *C. metapsilosis* was found not only *dadih*, but also in Chicha, a spontaneously fermented beverage, made from maize [43]. Yeasts and LAB grow simultaneously in the production of organic acids by LAB, which lower the pH of the medium, and may favor the growth of yeasts. While, yeasts provide the growth factors for the LAB, such as vitamins and components of soluble nitrogen [43]. That might be the reason why *C. metapsilosis* could survive and be found in *dadih*.

Table 2. Types of lactic acid bacteria found in *dadih*—fermented buffalo milk.

Region in West Sumatra	Types of Lactic Acid Bacteria	References
Bukittinggi and Padang Panjang	<i>Lactobacillus</i> sp., <i>Lactococcus</i> sp., and <i>Leuconostoc</i> sp.	[34]
Bukittinggi	<i>Lactococcus lactis</i> subsp. <i>lactis</i> , <i>Levilactobacillus brevis</i> , <i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i> , <i>Lacticaseibacillus casei</i> , <i>Lacticaseibacillus paracasei</i> , <i>Leuconostoc mesenteroides</i>	[13]
Lima Puluh Kota, Agam, Tanah Datar, Solok	<i>Levilactobacillus brevis</i> , <i>Weissella viridescens</i> , <i>Lentilactobacillus buchneri</i> , <i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i> , <i>Leuconostoc mesenteroides</i> , <i>Leuconostoc paramesenteroides</i> , <i>Streptococcus lactis</i> subsp. <i>diacetylactis</i> , <i>Streptococcus faecium</i> , <i>Streptococcus raffinolactis</i> , <i>Lactococcus piscium</i>	[44]
Payakumbuh	<i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i>	[45]
Sijunjung	<i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i>	[46]
Solok	<i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i>	[47]
Payakumbuh	<i>Lactobacillus</i> sp.	[48]
Palupuh	<i>Lactobacillus</i> sp., <i>Lactococcus</i> sp., <i>Leuconostoc</i> sp.	[40]
Gadut	<i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i> , <i>Lactococcus lactis</i> subsp. <i>cremoris</i> , <i>Lactococcus lactis</i> subsp. <i>lactis</i>	[39]
Kamang	<i>Lactiplantibacillus pentosus</i> , <i>Lactococcus lactis</i> subsp. <i>lactis</i> , <i>Pediococcus pentosaceus</i> , <i>Lactococcus lactis</i> subsp. <i>cremoris</i> , <i>Lactiplantibacillus plantarum</i> subsp. <i>plantarum</i>	[39]

6. Nutritional Value and Chemical Analysis of *Dadih*

The nutritional value and chemical analysis of *dadih* from different regions, as well as the developed *dadih* using different types of milk (see Section 9 for the backgrounds of developed *dadih*), was reported by previous studies, described in Tables 3 and 4, respectively. In Table 3, the nutritional value and chemical analysis of *dadih* were different from one region to another. For example, the pH and total acidity of *dadih* varied between regions. Helmizar et al. [9] reported that the type of buffalo feed may impact the pH and acidity of *dadih*. As an example, the grass (*Imperata cylindrica*) in Agam, and banto grass (*Leersia hexandra* Sw.) with rice straws in Tanah Datar were used to feed the buffaloes. These weeds contain several compounds such as malic and citric acid with different contents, which may affect the pH and acidity of the resulting *dadih*. Furthermore, the different types of bamboo (each type has different thickness, porosity, and other characteristics) used to produce *dadih* and the addition of food supplements given to the buffaloes in different regions may also affect the chemical composition of *dadih*, such as protein, fat, water content, etc. [9]. The uncontrolled production that causes different chemical compositions of *dadih* in different regions leads to the idea of production standardization, which is described in Section 9.

Table 3. Nutritional value of *dadih*—fermented buffalo milk from different regions in West Sumatra.

Chemical Characteristics of <i>Dadih</i>	Regions of West Sumatra				
	Agam	Sijunjung	Solok	Tanah Datar	
Protein (%)	10.89	7.06	5.01	6.91	12.41
Fat (%)	18.00	8.17	6.50	7.98	5.70
Carbohydrate (%)	8.03	ND	ND	ND	14.92
Water content (%)	61.94	82.40	75.45	81.79	66.09
Ash content (%)	1.14	0.91	0.68	0.92	0.72
pH	4.33	4.80	4.74	4.76	4.55

Table 3. Cont.

Chemical Characteristics of <i>Dadiah</i>	Regions of West Sumatra				
	Agam	Sijunjung	Solok	Tanah Datar	
Acidity (%)	1.70	1.28	1.17	1.32	0.51
Reference	[9]	[49]	[49]	[49]	[9]

ND: Not determined.

Some studies reported the substitution of buffalo milk using other types of milk as the substrate of *dadiah*. In this case, starter culture may be needed to start the fermentation process and to standardize the production process (see Section 9). The fat content of *dadiah* from buffalo milk and cow milk ranged from 5.70 to 18.00% (Table 3), and 7.03 to 10.96% (Table 4), respectively. Although the fat and total protein content of cow milk are lower than those of buffalo milk (Table 1), some additional processes, such as evaporation and addition of skim milk could increase the total solid content of cow milk, including fat and protein, to achieve the desirable *dadiah* properties [50]. The fat content of *dadiah* from soy milk is still lower than that from other kinds of milk, despite that skim milk was added to increase the total solid content.

Table 4. Nutritional value of developed *dadiah*—fermented milk made from different types of milk.

Chemical Characteristics of <i>Dadiah</i>	<i>Dadiah</i> from Other Types of Milk				
		Cow Milk		Goat Milk	Soy Milk
Starter culture (concentration)	<i>L. plantarum</i> + <i>L. acidophilus</i> + <i>B. bifidum</i> (3% w/v)	<i>L. plantarum</i> + <i>L. acidophilus</i> (3% w/v)	Back-slopping method using <i>dadiah</i> from Bukittinggi (4% w/w)	Spontaneous	Back-slopping method using <i>dadiah</i> from Bukittinggi (4% w/w)
Protein (%)	3.53	4.27	9.79	3.75	4.65
Fat (%)	10.96	7.59	7.03	ND	2.91
Carbohydrate (%)	ND	ND	ND	ND	ND
Water content (%)	83.15	73.72	ND	ND	ND
Ash content (%)	0.90	0.52	ND	ND	ND
pH	3.49	4.29	ND	6.54	ND
Acidity (%)	3.45	0.64	ND	0.31	ND
Reference	[51]	[52]	[53]	[54]	[53]

ND: Not determined; w/v: weight/volume; w/w—weight/weight.

7. The Functional Value of *Dadiah*

With a high amount of viable LAB, *dadiah* becomes a potential probiotic and provides many potential beneficial effects on health [10], including antimicrobial, immunomodulatory, antimutagenic, antioxidant, and hypocholesterolemic properties, and as a source of γ -aminobutyric acid (GABA) and folates.

7.1. Antimicrobial Properties

The decrease pH levels, competition for substrates, and the production of substances with a bactericidal or bacteriostatic action are several factors that result in the LAB from *dadiah* possessing antimicrobial activity [46]. *Lactobacillus* spp., which is the most superior probiotic found in *dadiah*, showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enteritidis* with inhibitory diameter 11.54 mm, 10.27 mm, and 16.31 mm, respectively [55]. *L. plantarum* strain 8m-21 isolates of *dadiah* from the Air Dingin region (Solok) exhibited antimicrobial activity against *E. coli* O157. The inhibitory zone of *L. plantarum* strain 8m-21 against *E. coli* O157 was 20.25 mm, greater than penicillin (2.70 mm), ampicillin (15.20 mm), and kanamycin (14.19 mm) as the controls after 24 h of incubation at 37 °C [10]. Furthermore, *L. plantarum* isolated from *dadiah* from the Sijunjung region showed 12 mm, 13.5 mm, and 12 mm of inhibitory zone against *E. coli*, *S. aureus*, and *S. typhi*, respectively, after 36 h of incubation [46]. Different antimicrobial activities of *L. plantarum* in *dadiah* originated from the Solok, Sijunjung, and Payakumbuh regions against *S. aureus*, *E.*

coli, and *S. typhi* were investigated, with *L. plantarum* in *dadih* from Payakumbuh showed the highest antimicrobial effect [56]. In *dadih* made from soy milk, *L. plantarum* strain BDL11 exhibited the highest bacteriocin activity against *Listeria monocytogenes* (inhibitory zone diameter 8 ± 0.02 mm) compared to other strains [57].

After 7 h of contact with *L. plantarum* from *dadih* from the Payakumbuh region, the death percentage of 33.3% of *S. aureus* colony, 27.27% of *E. faecalis* colony, and 23.07% of *E. coli* colony were reported, demonstrating that *L. plantarum* more effectively inhibits Gram-positive bacteria (*S. aureus* and *E. faecalis*) than Gram-negative bacteria (*E. coli*) [45]. This might be due to the outer membrane of Gram-negative bacteria, which functions as an efficient permeability barrier and is able to exclude macromolecules (i.e., bacteriocins or enzymes) and hydrophobic substances (i.e., hydrophobic antibiotics) [58]. Bacteriocin produced by *L. plantarum* is plantaricin that shows antimicrobial activity [59], including against *Staphylococcus* spp. [60]. The *L. plantarum* isolated from this *dadih* also survived in the concentration of 0.5% bile salts, and is resistant to acidic media until pH 2, which makes it a good probiotic that can inhibit the growth of pathogenic bacteria in the digestive tract [45].

Due to the beneficial antimicrobial properties from bacteriocin of LAB from *dadih*, it can be used as a natural preservative to prevent food-borne disease [61], including as a natural preservative of chicken meat [62].

7.2. Hypocholesterolemic Properties

The hypocholesterolemic activity of *dadih* was reported by previous studies. LAB in *dadih* may alter serum cholesterol by either directly binding to dietary cholesterol, deconjugation of bile salts, or in combination [63]. The bile salt hydrolase activity may contribute to the reduction of cholesterol, as the free bile salts (deconjugated) are less re-absorbable in the intestine compared to the conjugated bile salts, and may be excreted in the feces [13].

It was reported that *L. fermentum* I-11 and *Leuconostoc lactis* subsp. *lactis* I-2775 could be recommended as a probiotic to prevent coronary heart disease as those were tolerant to acid and ox gall (bile) and deconjugated sodium taurocholate and bound cholesterol [63]. Furthermore, taurocholate-deconjugating abilities were observed in *Lactococcus lactis* subsp. *lactis* IS-10285 and IS-29862 [13]. In rat studies, the rats fed with fermented kinds of milk made from *Lactococcus lactis* subsp. *lactis* IS-10285 from *dadih* exhibited significantly ($p < 0.05$) lower total bile acids in serum [12].

7.3. Antimutagenic Properties

The mechanism of the antimutagenic effect of LAB in *dadih* takes place because of the bonds between mutagens or carcinogens with the peptidoglycan contained in the LAB. Mutagens and carcinogens which are bound will be excreted via feces and urine [10].

The LAB isolated from *dadih*, such as *Leuconostoc paramesenteroides* R-62, R-8, *Streptococcus lactis* subsp. *diacetylactis* R-63, and *Streptococcus cremoris* R-48 were reported to have high antimutagenic activities toward various mutagens, such as N-nitroso-dimethylamine (NDMA), N-nitroso-diethylamine (NDEA), N-nitroso-piperidine (NPIP), and N-nitrosopyrrolidine (NPYR), which has led to the suggestion that some of these compounds may contribute to carcinogenesis in humans. Furthermore, the antimutagenic properties of milk cultured with LAB from *dadih*, especially *L. casei* subsp. *casei* R-52, against mutagenic terasi (traditional Indonesian shrimp paste), was observed [64]. Milk cultured with *Lactococcus lactis* subsp. *cremoris* R-48, *Leuconostoc mesentroides* R-51, and *Lactococcus lactis* subsp. *casei* R-68 isolated from *dadih* exhibited high inhibition against the mutagenicity of both heated salty and sweet tauco (traditional Indonesian saline paste made from fermented yellow soybeans) [65]. However, only *L. mesentroides* R-51 was tolerant to both acid and bile, and can be used as a probiotic in preventing the mutagenesis caused by mutagenic heated food like tauco [65]. In vivo antimutagenic properties of *E. faecium* IS-27526 isolated from *dadih* from Bukittinggi toward Trp-P1 were also observed [14].

7.4. Antioxidant Properties

Free peptides and amino acids that are released after milk protein breakdown possess antioxidant properties [66]. The antioxidant activity of cow milk *dadih* fermentation using 1% starter culture of *L. casei* and *L. plantarum* (ratio 1:1) using 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) method was observed [15]. It was stated that 72 h of fermentation in the making of cow milk *dadih* is the most optimum length of fermentation to receive the highest antioxidant activity [15]. The soluble protein of buffalo milk *dadih* (3 days of fermentation) originating from the Agam region showed higher antioxidant activities in Fe reducing power, scavenging activity against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and DPPH compared to those originated from the Sijunjung and Solok regions [67].

7.5. Immunomodulatory Properties

Several studies reported the immunomodulatory properties of LAB in *dadih*. The salivary secretory immunoglobulin A (sIgA) of underweight Indonesian preschool children was increased significantly ($p < 0.05$) in response to the *E. faecium* IS-27626 of *dadih* origin, supplemented at a dose of 2.31×10^8 cfu/day in 125 mL of ultra-high temperature (UHT) low-fat milk, after 90 days of supplementation [16]. Besides, weight gain of children with normal body weight was also observed, indicating that *E. faecium* IS-27626 may help in maintaining the integrity of the intestine, thus facilitating optimum nutrient absorption. The adhesion to and colonization of the mucosal surfaces are possible protective mechanisms against pathogens through competition for binding sites and nutrients or immune modulation [16].

A study reported the effect of *L. plantarum* IS-10506 of *dadih* origin (2.3×10^{10} cfu/day) with zinc supplementation (20 mg of zinc sulfate/day or 8 mg of zinc elemental/day) on humoral immune response in preschool children after 90 days of supplementation [68]. It was reported that fecal sIgA increased significantly in the probiotic group (30.33 ± 3.32 $\mu\text{g/g}$; $p < 0.01$) and in the combination probiotic and zinc group (27.55 ± 2.28 $\mu\text{g/g}$; $p < 0.027$), as compared with the placebo group (13.58 ± 2.26 $\mu\text{g/g}$). Furthermore, zinc supplementation can help to improve the zinc status of children [68]. In another study using *L. plantarum* IS-10506 from *dadih* as well, it was reported that the increasing production of sIgA in children younger than two years was observed, due to the stimulation of TGF- β 1 by *L. plantarum* IS-10506 supplementation. This result was associated with a significant correlation between TGF- β 1/TNF- α and fecal sIgA [69].

The sIgA serves as the first line of defense in protecting the intestinal epithelium from enteric toxins and pathogenic microorganisms. Via the immune exclusion process, sIgA promotes the clearance of antigens and pathogenic microorganisms from the intestinal lumen by blocking their access to epithelial receptors, entrapping them in mucus, and facilitating their removal by peristaltic and mucociliary activities [70]. A study in rats reported that *L. plantarum* Dad-13 contained in the combination with sweet potato fiber may have potency in systemic immune stimulation because of the tendency to increase the level of splenocyte IFN- γ in rats infected with *S. Typhimurium* [17].

7.6. GABA—Source

GABA (γ -aminobutyric acid) is the major inhibitory neurotransmitter in the mammalian central nervous system [71]. GABA, which effectively acts as a natural relaxant in humans, exists naturally in many kinds of foods at low levels, while higher levels could be found in fermented food products [72]. GABA was also reported to take part in preventing heat-induced stress in broiler chickens [73].

The LAB isolated from indigenous *dadih* from West Sumatra was reported as a potential producer of GABA, identified as *Pediococcus acidilactici* [19] and *L. plantarum* N5 [18]. The optimization of GABA production by *P. acidilactici* DS15 [74] and *L. plantarum* [18] were then investigated. The result showed that a higher amount of GABA production was found with the addition of 60 mM glutamate and 100% whey tofu and 15% palm sugar were

the best source of nitrogen and carbon, respectively, for *P. acidilactici* DS15 [74]. While the highest production of GABA by *L. plantarum* N5 was observed at pH of 5.5, at a temperature of 36 °C, glutamate concentration of 500 mM, and an incubation time of 84 h [18]. Furthermore, a study characterized the most productive GABA producing LAB, classified as *Lactobacillus* sp., in various indigenous fermented foods from West Sumatra, such as *dadih*, *ikan budu*, *asam durian*, and *tape/tapai*, which have anti-stress potential [75]. However, further research is needed to ensure the anti-stress activity of GABA producing LAB is isolated from *dadih*, especially in humans.

7.7. Folate—Source

Dairy products are good sources of folate for humans. Fermented milk products, especially yogurt, can contain a higher amount of folate [76]. In the human diet, folate is involved in many metabolic pathways, mainly in carbon transfer reactions such as purine and pyrimidine biosynthesis and amino acid interconversions [77]. A low intake of folate has been associated with Alzheimer's and coronary heart disease, osteoporosis, increased breast and colorectal cancer, poor cognitive performance, and hearing loss [77]. To date, the isolation and characterization of folate-producing LAB from *dadih* was reported. A total of 17 isolates from *dadih* were obtained and based on phenotype and genotype, 16 of them were identified as *L. plantarum* [20]. The folate production of the 17 selected isolates ranged from 12.43 to 27.84 µg/L, and folate production of *L. plantarum* Dad-13 as the control was 29.27 µg/L [20]. Further research related to the folate-producing ability of LAB from *dadih* is still needed.

8. *Dadih* in the Daily Diet

Dadih can be consumed directly or can be used in various dishes [22]. In Minangkabau, *dadih* is usually served during breakfast. *Dadih* is served with warm rice after adding the condiment, called sambal (ground or sliced shallot and chili) (Figure 3A), or it is added into *ampiang* (glutinous rice flakes), shaved ice, shredded coconut or coconut milk, and palm sugar syrup as the topping, which is commonly known as “*ampiang dadih*” [6,31] (Figure 3B). Sometimes, tapioca starch and ginger juice are also added to *ampiang dadih* [78].

To cook Minang beef curry (Figure 3C), many spices are added, including chili, coriander seeds, cumin, cinnamon, shallot, garlic, ginger, lemongrass, galangal, kaffir lime leaves, cloves, clove leaves, turmeric, and cardamom. Not only the spices, but coconut milk is also one of the main ingredients of beef curry. *Dadih* can be used as a substitute for coconut milk in beef curry [79].

Besides traditional dishes, *dadih* is also used in food product development. Some popular food products, such as jelly, beverages, ice cream, sandwiches, and cakes are also developed by using *dadih* as one of the main components or ingredients [22]. A study reported that ice cream containing *dadih* decreased the growth of *S. typhimurium* better than pure ice cream [80].

9. Challenges and Future Trends of *Dadih*

The main problem in *dadih* production is the decreasing buffalo milk production due to the decreasing buffalo population in West Sumatra [22,35]. Wirawati et al. [35] reported that in 2000, the total population of buffaloes in West Sumatra was 230,818 buffaloes based on Statistics of Sumatera Barat Provinces. In 2015, the population of buffaloes was 121,939 buffaloes [81], and in 2018, the number decreased again to 78,038 buffaloes [82]. The scarcity of buffalo milk may affect the price of buffalo milk and *dadih* [51]. To support the cultivation of buffalo, as well as the preservation of *dadih* production in West Sumatra, the West Sumatra Livestock and Animal Health Department initiated and controls a program to provide female buffaloes to local farmers and a buffalo artificial insemination program [83].

Besides the programs from the government, the researchers have studied the alternative ways to solve the limited source of buffalo milk as the main ingredient of *dadih*, as well as to improve the quality of *dadih*. The potency of alternative ingredients to alter the

limited buffalo milk in *dadih* production was studied, such as cow milk [35,50,53], goat milk [25,84,85], and soy milk [53,57] which are more available than buffalo milk. However, some additional processes, such as evaporation and skim milk addition, are needed to equal the total solid content of buffalo milk to produce *dadih* with desired properties [53,84,86].

As the sensory properties of *dadih* may differ due to various LAB used in fermentation and due to spontaneous fermentation in different regions, the protocol of *dadih* production and microbiota contributed in *dadih* need to be standardized to make *dadih* more significant and therefore safer for consumption [6,22]. Besides studying the potency of alternative milk, Taufik [51] reported that pasteurization, controlled fermentation, and changing of *dadih* containers from bamboo to alternative containers is more sterile and hygienic (e.g., plastic packaging) are also important to make *dadih* more significant in the food industry. Controlled fermentation of *dadih* from cow milk using a 3% starter combination of *L. plantarum*, *L. acidophilus*, and *Bifidobacterium bifidum* was reported [51]. Furthermore, changing packaging material from bamboo tubes to plastic tubes improved the sensory quality and shelf life of *dadih* made from buffalo milk [87]. However, the development of plastic packaging needs to be investigated, as plastic waste may cause environmental problems. To support the marketing of *dadih* as a functional food, a study reported on the effective design of *dadih* packaging, which is obtained based on positive opinion, complete labelling, ergonomics, and large font [88].

To explore the beneficial health properties of *dadih*, the *Dadih* Initiative was initiated in Indonesia in 2017 to establish and standardize specifications for production and quality control of *dadih*, therefore large quantities of *dadih* production for local people can be achieved [40]. *Dadih* powder was developed using freezing technology ($-20\text{ }^{\circ}\text{C}$, 2 days), followed by vacuum heating ($40\text{--}50\text{ }^{\circ}\text{C}$, 5–10 min) for powder stabilization. The nutritional composition of *dadih* powder was then standardized to be used as a complementary food to prevent children under two years from stunting in West Sumatra, Indonesia [89].

Another development was reported as *L. plantarum* Dad-13 isolated from *dadih* was successfully utilized as the starter culture in cheese production. However, it could not be categorized as probiotic cheese due to the low viability of *L. plantarum* Dad-13 in the resulting cheese [90]. Furthermore, a project developed an incubator for *dadih* production to accelerate the fermentation time [91]. The incubator was equipped with a digital thermometer to control the temperature during fermentation, and a stopwatch or timer to remind and inform the farmer that the fermentation process is done. By using this incubator at $40\text{ }^{\circ}\text{C}$, the fermentation time of *dadih* can be traditionally accelerated from 50 h to 5 h [91]. Furthermore, *dadih* was also involved in improving the nutritional quality (increased fat and protein content) of the cassava waste for animal feed within 10 days of the cassava waste fermentation process [92].

10. Conclusions

Buffalo became an important animal for the Minang people for hundreds of years, as every part of the buffalo is very useful to help local people, including as a source of nutrition. *Dadih* is traditional fermented buffalo milk of the Minangkabau, which is similar to yogurt and Indian *dahi*. However, they have different production processes, especially the usage of starter culture, heat treatment, production time, and composition of LAB. *Dadih* has been studied, whether in vitro or in vivo studies, to have potential health benefits. The indigenous LAB found in *dadih* has antimicrobial, hypocholesterolemic, antimutagenic, antioxidant, and immunomodulatory properties, as well as being a source of GABA (anti-stress agent) and folate formation. The indigenous microbes, including LAB and yeasts, found in buffalo milk and bamboo tubes also contribute to the formation of flavor and desired texture of *dadih*. Research is still ongoing to discover the potential health benefits and improve the sensory quality of *dadih*. However, many challenges were found in *dadih* production. The decrease in buffalo milk production due to the decreasing population of buffalo in the last two decades was observed. Furthermore, various properties of *dadih* were observed due to the spontaneous fermentation and the various compositions of microbes

in buffalo milk and bamboo tubes used in the fermentation. To preserve the production of *dadih* as the traditional food of the Minangkabau, the local government controls the buffalo cultivation program, while many researchers have studied alternative ways to find alternative milk sources, standardization, and specifications in the *dadih* fermentation process, and quality control of *dadih*. With various types of development, *dadih* will be more significant in the food industry.

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Abbreviations

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); cfu: colony forming unit; DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate; GABA: γ -aminobutyric acid; LAB: lactic acid bacteria; sIgA: secretory immunoglobulin A; UHT: ultra-high temperature.

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