Fatty acid composition of fermented buffalo milk (*dadih*) as traditional probiotics

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Abstract. *Dadih* is a fermented food containing bacteria that transform buffalo milk properties. The lactic acid bacteria (LAB) in *dadih* contribute to health promoting effects, including dietary fatty acid production. This research aims to assess the fatty acid distribution, total LAB, and antioxidant capacity of *dadih*. Samples were obtained from buffalo cattlefarmers and analyzed in two groups: fresh and microwave-heated *dadih*. Both samples were tested for fatty acid content, total LAB, and antioxidant capacity. The research findings showed an average fat content of 7.13% in fresh *dadih* and 7.23% in heated *dadih*. The heated *dadih* has a different fatty acid profile. The linoleic acid, omega-6, and omega-9 content increase due to heated *dadih*, whereas the omega-3 and eicosapentaenoic acid decrease slightly due to heating processes. The primary composition of dadih is palmitic and oleic acids, which are the most abundant fatty acids present. The antioxidant capacity of heated *dadih* was 6.13% lower than the 37.65% found in fresh *dadih*. The average total LAB counts 8.00 in fresh *dadih* and 7.74 log cfu/g in heated *dadih*. The microwave heating preserved the fatty acid composition but decreased the viability of LAB and negatively impacted certain antioxidant components.

1 Introduction

The microbial composition of *dadih*, a probiotic milk product originating from West Sumatra, Indonesia, is characterised by the presence of *Lactococcus, Lactobacillus, Bifidobacterium, Streptococcus,* and *Leuconostoc* species [1]. Probiotics consumption positively modulates the gut microbiota. These probiotics are considered a promising therapeutic approach to metabolic syndrome [2]. A number of chronic diseases, including type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), coronary heart disease (CHD), obesity, and hypertension, have been linked to gut dysbiosis [3]. To qualify as probiotics, bacteria must

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be viable, present in adequate numbers, able to withstand low pH and organic acid, and provide health benefits. These bacteria convert lactose into lactic acid, produce metabolic compounds such as exopolysaccharides, peptides, and fatty acids, while also serving as fermentation starters.

Fermentation with a mixed starter culture efficiently produces fatty acids composition compared to a single starter. Metabolites generated during fermentation, such as fatty acids, show potential as antioxidants, and the intake of some polyunsaturated fatty acids (PUFA) positively correlates with plasma antioxidant parameters [4]. Key microbial metabolites are organic acids, enzymes, amino acids, vitamins, pigments, and bioactive compounds, such as polyphenolics and alkaloids. These compounds improve flavor and nutritional value while providing health benefits, including antioxidants, antimicrobial, and probiotic activities [5,6].

Heating can inactive probiotic cells, by targeting the peptidoglycan cell wall, leading to cell lysis; the nucleoid, resulting in DNA demage; cellular RNA and ribosomes, imparing protein synthesis; and various enzymatic system, distrupting metabolic process. Heat treatment led to the loss of D-alanine in the peptidoglycan wall of the probiotics, compromising cell wall integrity. Additionally, heating impacts the nutritional value of food by increasing moisture content, which reduces dry matter, protein, fat, ash, carbohydrates, and caloric content. PUFA and *trans* fatty acids are more heat-sensitive compared to saturated fatty acids. Therefore, foods rich in PUFA and low in saturated fatty acid should undergo minimal heating to preserve their nutritional quality [7].

Recently, *dadih* has been consumed fresh, but it's short shelf life limits its usability. The limited variety of processed *dadih* products is primarily due to the widespread use of heating processes in food production, which reduces probiotic activity. However, *dadih* has potential as a derivative product with greater commercial value. Additional information is needed to understand the fatty acid distribution of both fresh and heated *dadih*. Therefore, this research aims to evaluate the fatty acid distribution, total LAB count, and antioxidant capacity in both fresh and heated *dadih*.

2 Materials and methods

2.1 Materials

This research utilized *dadih* sourced from buffalo cattle farmers in Kamang, Agam Regency, West Sumatra, Indonesia. Fatty acid analysis was conducted at SIG Laboratory. Materials used included buffered peptone water (BPW), de Man Ragosa-Sharpe (MRS) agar, and distilled water for the viable LAB count. The antioxidant capacity was determined by employing a DPPH (2,2-diphenyl-1-picryl-hydrazylhydrate) solution, ethanol 95% (v/v), and distilled water were utilized.

The equipment used for heating *dadih* included a *Sharp-R21a1(W)In* microwave. Analytical tools included an analytical balance, micropipette, vortex, glass tools, spectrophotometer, and Gas Chromatography Clarus® 580 GC-FID.

2.2 Dadih preparation

Buffalo milk was fermented traditionally in bamboo sticks for 48 hours to produce *dadih*. The *dadih* was divided into two groups: fresh *dadih* and heated *dadih*. Heating was performed using a microwave (400 W, 77 seconds) [8,9]. Both groups were packed in plastic pouches, labeled, and stored for 24 hours in a container with a thermal freeze ice gel pack. All samples were collected and sent to the SIG laboratory for analytical testing.

2.3 Fatty acid content

The 18-6-1/MU/SMM-SIG method was utilised for the analysis of fatty acid in the *dadih* samples. The instrument is equipped with Gas Chromatography accompanied by a flame ionization detector (FID). The procedure began with dissolving one gram of each *dadih* sample in hexane, followed by 0.1 ml of 2N KOH and isopropyl alcohol. The mixture was heated for 1 hour at 45°C in a water bath. Following saponification, the mixture was thoroughly agitated with 1 ml of hexane using a mechanical shaker. Distilled water was added to the mixture, and subsequently, a centrifugal force was applied. A 1µl alliquot of the fatty acid methyl ester (FAME) was injected into the GC. The GC was operated at 220°C for the injection and 250°C for the detector. Nitrogen gas (N_2) was used at 1.5 ml/min as the mobile phase.

2.4 Total plate count

The enumeration of LAB colonies was performed according to the ISO 15214:1998 procedure. One gram of *dadih* was diluted in a BPW medium and homogenized using a vortex. The solution was serially diluted, and each dilution was pour-plated on MRS agar. The plates were incubated for 72 hours at 30°C for 72 hours. The enumeration of the colonies wes counducted, and the CFU was calculated. Total colony counts were performed on Petri dishes containing between 15 and 300 colonies.

2.5 Antioxidant capacity

The DPPH (2,2-diphenyl-1-picryl-hydrazylhydrate) assay was utilized for the determination of antioxidant capacity. A mixture was prepared by combining five grams of *dadih* with 50 ml of a solution composed of equal volume of ethanol and distilled water. Fifty mg/l DPPH was added to the solution, thoroughly shaken, and allowed to stand at room temperature. The calculation of the percentage inhibition of DPPH oxidation was performed as follows:

$$\%inhibition = \frac{(abs\ control - abs\ sample)}{abs\ control} \times 100$$

A UV-VIS spectrophotometer was employed to measure the absorbance at 517 nm.

2.6 Statistical analysis

This study employed a descriptive quantitative research design. Quantitative descriptive data were used to describe, explain, and predict the phenomena studied in this research utilizing statistical or numerical data. All data are reported as the mean of two measurements.

3 Results and discussion

The fat content was 7.13% in fresh *dadih* and 7.23% in heated *dadih*. The higher fat content in the heated *dadih* is likely due to the heating process, which reduces moisture and increases the relative fat content. Table 1 presents the data for the fatty acid composition of dadih.

Table 1. Fatty acid distribution in fresh *dadih* compared to heated *dadih*

Saturated fatty acid	**Fatty acids (g/100g of fat)**		**Unsaturated fatty acid**	**Fatty acids (g/100g of fat)**	
	Fresh *dadih*	**Heated *dadih***		**Fresh *dadih***	**Heated *dadih***
C4:0	2.34±0.04	2.59±0.00	C14:1	0.64±0.01	0.58±0.01
C6:0	1.48±0.00	2.13±0.71	C15:1	0.44±0.01	0.00±0.00
C8:0	0.74±0.01	0.79±0.01	C16:1	1.60±0.01	1.56±0.01
C10:0	1.51±0.06	1.50±0.01	C17:1	0.35±0.01	0.36±0.01
C11:0	0.03±0.00	0.03±0.01	C18:1 cis	27.18±0.02	27.69±0.01
C12:0	2.25±0.08	2.10±0.01	C20:1	0.10±0.00	0.00±0.00
C13:0	0.13±0.00	0.10±0.00	C22:1 ω9	0.00±0.00	0.00±0.00
C14:0	11.88±0.16	11.58±0.01	C24:1	0.00±0.00	0.00±0.00
C15:0	1.87±0.01	1.74±0.04	**Total MUFA**	30.19±0.06	30.18±0.04
C16:0	30.82±0.23	31.72±0.04	C18:2 ω6c	0.85±0.01	0.94±0.02
C17:0	1.29±0.01	1.22±0.00	C18:3 ω3	0.35±0.00	0.36±0.00
C18:0	12.41±0.01	12.64±0.00	C20:2	0.00±0.00	0.14±0.00
C20:0	0.25±0.01	0.27±0.01	C20:3 ω6	0.00±0.00	0.03±0.00
C21:0	0.06±0.00	0.05±0.00	C20:4 ω6	0.07±0.00	0.08±0.00
C23:0	0.10±0.00	0.08±0.00	C20:5 ω3	0.15±0.00	0.14±0.01
C24:0	0.14±0.01	0.13±0.00	C22:2	1.03±0.01	0.00±0.00
Total SFA	67.27±0.62	68.65±0.84	**Total PUFA**	2.45±0.02	1.68±0.04

Note: All displayed result are expressed as mean±SD (n=2)

This study analysis revealed that palmitic acid (C16:0) constituted the highest proportion of saturated fatty acids, whereas oleic acid (C18:1) was the most abundant unsaturated fatty acid. Linolenic acid (C18:3), omega-6, and omega-9 showed an increasing trend. In contrast, omega-3 and eicosapentaenoic acid (EPA) decreased in heated *dadih*. *Dadih* is characterised by a high content of palmitic and oleic acids. The predominant fatty acids in both fresh and heated dadih are palmitic acid (C16:0) and oleic acid (C18:1). Palmitic acid is a common and significant fatty acid found in dairy product [10]. Both palmitic acid and oleic acid play essential roles in health. Palmitic acid supports product stability during storage. However, excessive consumption should be avoided as it can increase LDL cholesterol levels. In contrast, oleic acid positively impacts heart health by reducing LDL cholesterol and increasing HDL cholesterol, making it a beneficial cardiovascular fat [11].

This study found that linoleic acid levels in *dadih* increased after heating compared to fresh *dadih*. Linoleic acid, an omega-6 polyunsaturated fatty acids, is necessary for supporting cardiovascular health and cellular function. The increase in linoleic acid concentrations may result from changes in fat structure during heating, which reduces specific fat components while relatively increasing linoleic acid levels [11]. A high intake of linoleic acid provides significant health benefits, supporting cell membrane function and reducing the risk of cardiovascular disease and inflammation. The elevated linoleic acid levels observed in *dadih*, especially after heating, indicate the product's potential as a valuable source of beneficial fatty acids that support cardiovascular health and optimize bodily function [12].

The classification of fatty acids into saturated and unsaturated categories is based on the structure of their carbon chains. Saturated fatty acids possess only single bonds in their carbon chains and have capacity to bind two hydrogen atoms. Unsaturated fatty acids are distinguished by the presence of at least one double bond. Unsaturated fatty acids are classified as monounsaturated (MUFA) or polyunsaturated (PUFA) based on their double bonds. A high content of MUFA and PUFA in food can positively affect health.

The fatty acid composition of dadih shows specific characteristics that can be influenced by the heating and fermentation process. The concentration of saturated fatty acid in dadih tends to increase after heating, as the heating process reduces moisture content and some unsaturated fatty acid components that are more sensitive to high temperatures. This results in a relative increase in the concentration of saturated fatty acids. Palmitic acid remains stable during heating and is dominant in the saturated fatty acid profile [13].

The total LAB in both fresh and heated *dadih* suggests that it can serve as a probiotic food source. The average total LAB in fresh and heated *dadih* were 8.00 and 7.74 log cfu/g, respectively. The antioxidant activity of heated *dadih* was 6.13%, lower than to the 37.65% found in fresh *dadih*. The microwave heating process showed that the fatty acid composition remained relatively unchanged. Furthermore, this study reported a decrease in both LAB and antioxidant activity in heated *dadih*. The incorporation of encapsulants into fermented milk is an effective strategy to prevent LAB viability from thermal damage while maintaining the stability of antioxidant compounds.

The utilisation of microencapsulation techniques with materials such as alginate, chitosan and gellan gum has been shown to enhance thermal resistance, inhibit heat transfer, and reduce cell membrane permeability, protein denaturation, and the inactivation of LAB metabolic enzymes. Antioxidants like gallic acid, catechin, and quercetin are protected from degradation by microcapsules, shielding them from milk proteins and acidic conditions. The gradual release of antioxidants helps maintain stability and protect lactic acid bacteria (LAB) from stress during heating [14].

The optimal growth temperature for LAB is 30-40°C. During heating processing, exposure to high temperatures decreases the viability of bacteria and damages some sensitive antioxidant components. These LAB play a role in shaping the fatty acid profile of fermented buffalo milk. The increase in linoleate (C18:2) may result from the hydrogenation of oleic acid (C18:1) facilitated by LAB. The presence of linoleate in *dadih* contributes to its potential antioxidant properties [15].

4 Conclusion

The microwave heating preserves the fatty acid profile of *dadih* while slightly increasing total fat content due to moisture reduction. Palmitic acid and oleic acid remain the most abundant fatty acids in both fresh and heated *dadih*. Heating increases linoleic acid (omega-6) and omega-9 levels but slightly decreases omega-3 and eicosapentaenoic acid (EPA), suggesting that minimal heating is preferable to maintain these sensitive fatty acids.

However, the treatment resulted in a reduction of LAB and antioxidant activity. Despite these reductions, heated dadih retains essential nutrients and beneficial fatty acids, supporting cardiovascular health. Therefore, incorporating an encapsulant during the heating process could help preserve these properties in *dadih*.

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