Quality and storage time of traditional dangke cheese inoculated with indigenous lactic acid bacteria isolated from Enrekang District, South Sulawesi, Indonesia

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Manuscript received: 22 April 2022. Revision accepted: 30 May 2022.

Abstract. Zakariah MA, Malaka R, Laga A, Ako A, Zakariah M, Mauliah FU. 2022. Quality and storage time of traditional dangke cheese inoculated with indigenous lactic acid bacteria isolated from Enrekang District, South Sulawesi, Indonesia. Biodiversitas 23: 3270-3276. Dangke is a traditional cheese from Enrekang District. Many research on the isolation of lactic acid bacteria (LAB) from dangke cheese has been done without knowing the type of LAB species. The aim of this study was to find out the type of LAB species that have been successfully isolated and applied to dangke cheese. The design of this study was conducted in two stages: 1) isolated LAB species were identified by 16S rRNA sequencing; 2) effect of inoculated LAB on the quality and storage time of traditional dangke cheese. The molecular results showed that LAB from dangke cheese inoculated Streptococcus lutetiansis, Weissella confusa and Streptococcus equines. Isolates type and ripening time have affected pH, organic matter, and dangke cheese crude fat. The type of isolate can partially affect the dry matter content, and dangke cheese crude protein. Weissella confusa can improve the quality of dangke with ripening time of 6 days and a storage time of 3 days.

Keywords: Cheese, dangke, LAB species, Streptococcus lutetiansis, Weissella confusa, Streptococcus equines

INTRODUCTION

Milk is a very important food in human life because it is the main food after the birth of mammals. With the development of increasingly advanced technology, efforts to process milk into various dairy products (diversification of dairy products) are growing. Various efforts have been made by the milk processing industry and researchers to increase product durability and improve product quality. Fresh milk can be made into a variety of processed products, including pasteurized milk, sterilized milk, ice cream, yogurt, powdered milk, and cheese.

Indonesia is the largest archipelagic country that is bestowed by God with a lot of traditional foods, one of which is traditional milk food from milk. Three types of traditional foods made from milk are types of butter, yogurt, and cheese. Dairy products that have various types of cheese according to their regional origin, such as dali or bagot ni horbo in Tapanuli, Cologanti and Litsusu in Nusa Tenggara, Dangke in Enrekang (South Sulawesi).

Dangke is a product from milk, coagulated (to be curd) by the sap of papaya (contain papain enzyme), it is made from coconut shell, and packaging by banana leaf. The name dangke was derived from “dank u wel”, which is Dutch means thank you very much, according to local stories, natives presented this product to the Dutch on their first visit to South Sulawesi and subsequently said “dank je”, a shortened from of “dank u wel”, today the local called dangke.

Papaya stems, leaves and fruit contain white sap, in papaya sap there are more than 50 amino acids including aspartate, threonine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, triptophan, and cysteine. In addition, the sap also contains an enzyme that breaks down proteins or proteolytic enzymes called papain. Papain enzyme which is a coagulation factor for milk protein in the manufacture of dangke.

All dangke workers in Enrekang Regency use coconut shells as a tool for making dangke, the remaining liquid from the initial filtration will come out through the holes at the bottom of the shell, the lumps are molded in hot conditions so that they can stick together so that the resulting dangke texture is dense and compact. Dangke is generally wrapped in banana leaves, leaf length, leaf width, leaf thickness, as well as the number, diameter and tensile strength of banana leaf fibers are supporting factors so that
banana leaves become a potential material for wrapping food.

Chemical composition of dangke from dangke producer in Enrekang were water 58.28%, ash 1.83%, crude protein 15.57%, fat 20.71%, carbohydrate 3.60%.(Zakariah et al. 2019). Hatta et al. (2013) showed that compositional dangke from the producer were water 55%, ash 2.10%, crude protein 23.80%, and fat 14.80%.

Dangke is likely the source of lactic acid bacteria (LAB), because it is made from milk. Identification of bacterial RNA from LAB becomes an accurate technique in providing isolate information isolated from dangke, rather than macroscopic identification (shape, number, elevation, margins, color and sensitivity of the colony), microscopic (shape and gram reaction), and biochemical (product fermentation).

Previous studies conducted by other researchers mentioned that lactic acid bacteria obtained from dangke with molecular identification are Pediococcus acidilactici (Putra 2015), Lactobacillus delbrueckii subsp. lactis (Jimnoko et al. 2017), Limosilactobacillus fermentum (Syah et al. 2017), Lactiplantibacillus plantarum subsp. plantarum (Nur et al. 2017). Dangke was inoculated by Lactiplantibacillus plantarum subsp. plantarum (FNCC 0086) storage time is 2nd day on room temperature (Arini 2016). Therefore, this study aims to determine which isolate species have been successfully isolated from dangke, and measure quality and storage time on room temperature.

**MATERIALS AND METHODS**

The research was conducted in July 2019, taking samples of 4 selected isolates from previous studies. The test was conducted at the PCR Laboratory of the Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar. The research was conducted in two stages: 1) isolated LAB species were identified by 16S rRNA sequencing; 2) effect of inoculated LAB on the quality and storage time of traditional dangke. The LAB isolates were identified by the following steps: 1) DNA extraction; 2) gene amplification 16S rRNA by polymerase chain reaction; 3) agarose gel electrophoresis; and 4) sequencing gene (Syah 2018). Data analysis and species identification, the nucleotide sequences of the 16S rRNA genes from all LAB isolates were analyzed and identified using GenBank data library and BLAST (Basic Local Alignment Search Tool) Program on the NCBI website http://blast.ncbi.nlm.nih.gov/Blast.cgi.

LAB isolates were inoculated on dangke (Sabil et al. 2017), which was then divided into two groups. In group 1 inoculated dangke was stored at room temperature; and in group 2 dangke inoculated with LAB was stored in a refrigerator at 10°C in 4x4 design (factor A was the type of isolated LAB, factor b was storage time i.e. 0, 3, 6, and 9 day), pH and total titrated acid was calculated according to (AOAC 2005); hardness and total plate count was measured as described by (Malaka 2016; Rati et al. 2017). Proximate analysis include water content, ash, crude protein, and crude fat (AOAC 2005). Data analysis by regression linear model were executed by SPSS version 21.0 (Soleh 2005).

**RESULTS AND DISCUSSION**

16S rRNA gene sequence of lactic acid bacteria isolated from dangke cheese

The result of 16S rRNA PCR electrophoresis study is presented in Figure 1.

Lactic acid bacterial isolates obtained from dangke were identified by 16S rRNA gene sequencing. Four samples were selected for the Polymerase Chain Reaction (PCR). All 4 isolate samples had 1500 bp (base pair). Putri et al. (2018) noted that the 16S rRNA gene is most widely used as a molecular marker to determine bacterial species, 16S rRNA gene size is around 1500 bp.

The results of sequencing analysis showed that lactic acid bacteria derived from dangke include Streptococcus lutetiensis, Weissella confusa, and Streptococcus equines. Streptococcus being the most common genus found in this study consisted of Streptococcus lutetiensis strain TSGB1255 and Streptococcus equines strain TSGB1230 (Table 1). This was also reported by Medeiros et al. (2016) that the genus Streptococcus is the largest genus found in cheese. He identified Streptococcus infantarius subsp. infantarius, Streptococcus lutetiensis, Streptococcus macedonicus and Streptococcus waiu. Streptococcus lutetiensis is also found in the Italian cheese "Vastedda della Valle Del Belice” and armola cheese produced in Turkey (Gaglio et al. 2014; Orsahin 2012). Ayeni et al. (2011) reported that Weissella confusa is used as a Nigerian traditional fermented food and acts as a probiotic. Di Cagno et al. (2006) reported that Weissella confusa is found in 4 types of Italian cheese (Casciotta di Urbino, Barricato San Martino, Vento d’Estate, and Ubriaco di Rabosso).

Effect of inoculated LAB and ripening time on dangke quality

Inoculation with isolated LBA and varied ripening duration altered the physical quality of dangke pH (P <0.05), inoculation lowered the pH, thus the ripening time of dangke was 9 days (Figure 2). Dangke control (which was not isolated with LAB) has a high pH, so high pH values in dangke can disrupt the experiment. The usage of dissolved carbohydrates in dangke caused the pH to drop. Indigenous LAB isolates required water soluble carbohydrates to carry out metabolic reaction.

Ranjit and Kung (2000) stated that there is a positive correlation between water-soluble carbohydrates and pH. Dangke pH range was in the normal range of pH standards for dairy products (Badan Standarisisasi Nasional 1992). Soda et al. (2003) noticed that RAS cheese inoculated with LAB had a lower pH than cheese without inoculation. Inoculation and ripening time did not have a significant effect on the total titrated acid (Figure 3).

The results of the level of hardness of inoculated dangke is shown in Figure 4. Inoculation had a significant
effect on the level of hardness, but different ripening times had an effect on the hardness levels of dangke (P<0.05). Jaster et al. (2018) reported that inoculation with *Geotrichum candidum* on soft cheeses with a ripening time of 1, 7, 14 and 21 days had a significant effect on the level of hardness. This indicates that the longer the ripening time the lower the hardness level.

Dangke injected with indigenous LAB isolate had a higher dry matter than dangke that had not been inoculated with isolate (P<0.05). However, ripened time had no effect on dry matter (Figure 5). The high dry matter of dangke inoculated with LAB did not increase dramatically, but the use of dry matter (DM) was more efficient and effective in the biochemical process of fermentation with LAB inoculation on dangke, whereas dangke without inoculation allowed bacteria from other sources to use dry ingredient of dangke. Zakariah et al. (2015) stated that DM content with LAB inoculation have a higher DM than without inoculation. This is also supported by Soda et al. (2003) who reported that LAB inoculation produced a higher dry matter content than RAS cheese without inoculation.

The result of crude protein of dangke is shown in Figure 6. Crude protein (CP) in dangke inoculated with indigenous LAB was higher than dangke without inoculation (P<0.05), while ripened time had no effect on crude protein. Inoculation of *W. confusa* and *Streptococcus equinus*, and *Streptococcus lutetiensis* resulted in higher CP content of dangke than dangke without inoculation. The increase in CP was due to the biochemical process of fermentation. Hwang and Lee (2018) observed that inoculation of *W. confusa* in kimchi resulted in an increase in the amino acid content of citrulline and ornithine. Lorusso et al. (2018) reported that inoculation of *W. confusa* on the substrate increase the free amino acid with increasing fermentation time.

Crude fat (CF) of dangke was influenced (P<0.05) by indigenous LAB isolates and ripening time (Figure 7). Dangke inoculated with *W. confusa* had higher CF content than dangke without inoculation. This is due to lipolysis activity which causes an increase in the free fatty acid content. Jin et al (2019) reported that lipolysis process occurs in *W. confusa* isolates. An increase in ripening time leads an increase in the LK content in dangke. Soda et al. (2003) noticed that an increase in ripening time resulted in an increase in the free fatty acid content of RAS cheese inoculated with LAB compared to RAS cheese without inoculation.

Prolonged ripening results in an increase in the total number of LAB in MRSA media, this is in accordance with the opinion of Kesuma et al. (2013) who reported that total microbes in dangke increased over time. The ripening temperature was 10°C, so that some bacteria that were not mesophilic could not grow. Effect of LAB and ripening time on the total plate number are presented in Figure 8.

**Effect of inoculated LAB on the storage time of dangke**

The microbiological condition of dangke with a ripening time of 9 days has passed the threshold set out in Appendix O of the Department of Defense Food Safety and Quality Assurance Action Level (2009), that the maximum limit for dairy products (solid-semisolid) does not exceed 10 Log CFU/g. Therefore, the ripening time of 6 days can be the priority choice in making dangke. At the ripening time of 6 days, results showed that dangke inoculated with *S. luteinis* and *W. confusa* had higher LAB level than the other treatments and controls. Figure 9 showed the effect of inoculation on pH and total titrated acid during the storage time (at room temperature 30°C) of dangke.

Dangke inoculated with LAB had a lower pH than the control dangke (P<0.05). That was related to content of total acid titration on dangke. The pH of dangke exhibited a significant difference (P<0.05), as shown in Figure 10. Rohana (2018) also discovered that the longer the storage time of buffalo milk cheese, the lower pH. The decrease in pH with increasing time describes the addition of acid to the dangke. This is evidenced by the addition of acid to the total dangke titrated acid which was influenced (P<0.05) by inoculation and storage time (Figure 10).

**Table 1.** PCR sequencing of four isolates

<table>
<thead>
<tr>
<th>Samples</th>
<th>Description</th>
<th>Total Score</th>
<th>Query Cover</th>
<th>E. Value</th>
<th>Percent Identity</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASK1</td>
<td><em>Streptococcus lutetiensis</em> strain TSBG1255</td>
<td>1628</td>
<td>99%</td>
<td>0.0</td>
<td>98.69%</td>
<td>MN255551.</td>
</tr>
<tr>
<td>ASK2</td>
<td><em>Weissella confusa</em> strain qtm45</td>
<td>928</td>
<td>99%</td>
<td>0.0</td>
<td>97.94%</td>
<td>MN394833.1</td>
</tr>
<tr>
<td>ASK3</td>
<td><em>Streptococcus equinus</em> strain TSBG1230</td>
<td>2084</td>
<td>99%</td>
<td>0.0</td>
<td>97.11%</td>
<td>MN255526.1</td>
</tr>
<tr>
<td>ASK4</td>
<td><em>Streptococcus equinus</em> strain TSBG1230</td>
<td>1845</td>
<td>99%</td>
<td>0.0</td>
<td>95.66%</td>
<td>MN255526.1</td>
</tr>
</tbody>
</table>

**Figure 1.** PCR electrophoresis
Figure 2. Effect of inoculation and ripening time on the pH of dangke

Figure 3. Effect of inoculation and ripening time on total acid titration

Figure 4. Effect of inoculation and ripening time on the level of dangke hardness

Figure 5. Effect of inoculation and ripening time on the dry matter of dangke

Figure 6. Effect of inoculation and ripening time on the crude protein of dangke

Figure 7. Effect of inoculation and ripening time on the crude fat of dangke

Figure 8. Effect of inoculation and ripening time on total plate count

Figure 9. pH dangke at different storage time
The pattern of decreasing pH started from day 1 to day 3, and pH increased from day 4 to day 5 (Figure 11). According to Rohana (2018), a decrease in pH makes the texture brittle and easy to crumble, while an increase in pH makes the cheese texture elastic.

The results showed that there was a downward trend till the 3rd day and then increased on the 4th day till the 5th day of storage. The increase in the hardness levels on dangke on the 4th and 5th day could probably be due to the presence of fungus. The fungal growth was indicated by physical observation of dangke which contains hyphae. The pattern was polynomial trend pattern. Rohmatussollihat et al. (2015) reported the calculation of optimization of curd production in the manufacture of mozzarella cheese with a regression equation with a polynomial trend pattern.

Fungal hyphae appear on the surface of dangke on 4th and 5th day of storage time due to evaporation of water. Buckle et al. (2007) stated that the influence of room temperature and storage time greatly determines the growth of fungi, depending on the availability of nutrients and water sources for growth substrates in the media.

The hardness of cheese is affected by the water content in it. The matrix has a higher water content and has a soft texture (Noronha et al. 2008). Madadlou et al. (2007) reported that homogenization process can improve the texture, functional properties, sensory (flavour, appearance, overall acceptability), and yield of the resulting cheese. El-Bakry et al. (2001) stated that high hardness of low-fat cheese can be reduced by reducing the concentration of NaCl during cheese making. The total number of plates begins to enter the logarithmic phase during the storage period of 3rd to 4th days (Figures 12 and 13). The total number of plates in dangke with different coating types (carrageenan, agar, and bee wax) showed that the storage time from 0 (3.95 log CGU/ml) to 3 days (4.56 log CFU/ml) resulted in a significant increase (Soraya 2016).

Another thing was observed that the storage time of dangke with LAB inoculation could only last for 3 days. This is because the storage time of 4 days has passed the microbiological threshold set by the Appendix O Department of Defense Food Safety and Quality Assurance Action Level (2009), that the maximum limit for dairy products (solid-semisolid) does not exceed 10 log CFU /g.

Figure 10. Total titration acid dangke inoculated at different storage time

Figure 11. The level of hardness of dangke inoculated at different storage time

Figure 12. Total plate count (NA medium) of dangke inoculated at different storage time
In conclusion results of 16S rRNA sequencing revealed that LAB isolated from dangke contains *Streptococcus lutetiensis*, *Weissella confusa* and *Streptococcus equines*. All 3 isolates, namely *Streptococcus lutetiensis*, *Weissella confusa*, and *Streptococcus equines*, have similar effects on the quality and storage time of dangke, which is as follows:

(i) Application of dangke without inoculation has low hardness (0.47 kg/cm2), high acidity value (pH 6.34), low total acid (0.48%), low dry matter content (36.04%), high organic matter (97.24%), high crude protein (18.93%), high crude fat (13.05%) with a storage time of 3 days at room temperature.

(ii) Application of dangke with *Streptococcus lutetiensis* inoculation has high hardness (0.50 kg/cm2), low acidity value (pH 6.19), high total acid (0.52%), high dry matter (44.80%), low organic matter (97.24%), high crude protein (18.93%), high crude fat (13.50%) with a storage time of 3 days at room temperature.

(iii) Application of dangke with *Weissella confusa* inoculation has low hardness (0.45 kg/cm2), low acidity value (pH 6.19), high total acid titration (0.58%), high dry matter (46.89%), low organic matter (97.03%), high crude protein (21.55%), high crude fat (13.55) with a storage time of 3 days at room temperature.

(iv) Application of dangke with *Streptococcus equines* inoculation has a level of hardness (0.55 kg/cm2), low acidity value (pH 6.12), high total acid titration (0.54%), high dry matter (49.35%), low organic matter (97.01%), high crude protein (20.47%), high crude fat (13.94%) with a storage time of 3 days at room temperature.

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